PHARMACEUTICAL INNOVATION AFTER WORLD WAR II: FROM RATIONAL DRUG DISCOVERY TO BIOPHARMACEUTICALS

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PHARMACEUTICAL INNOVATION AFTER WORLD WAR II: FROM RATIONAL DRUG DISCOVERY TO BIOPHARMACEUTICALS

Topic Editors:
Apostolos Zarros, Queen Mary University of London, United Kingdom
Tilli Tansey, Queen Mary University of London, United Kingdom

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EDITORIAL
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Editorial: Pharmaceutical Innovation After World War II: From Rational Drug Discovery to Biopharmaceuticals

Apostolos Zarros and Tilli Tansey**

History of Modern Biomedicine Research Group, School of History, Queen Mary University of London, London, United Kingdom

Keywords: pharmaceutical innovation, history of pharmacology, drug discovery, biopharmaceuticals, drug repurposing, twentieth century, pharmaceutical industry

Editorial on the Research Topic

Pharmaceutical Innovation After World War II: From Rational Drug Discovery to Biopharmaceuticals

The late nineteenth century witnessed unprecedented advances in biomedical sciences, with the promotion in particular of physiological experimentation, bacteriological investigation, and chemical research (Bynum, 1994). These fields expanded and diversified after World War II contemporaneously with technological innovations, increased educational opportunities, the expansion of communication networks, and the creation of a wide range of healthcare markets, from private commercialized arrangements to nationalized organizations. Concomitant with and reflective of these changes were increased expectations of health and wellbeing, and the belief that medical advances could prevent, alleviate, treat, and/or cure physical and mental ills (Hardy and Tansey, 2006).

In the period under review, we have seen, *inter alia*, the shift from traditional *materia medica* to laboratory- and industry-based research; the establishment of the chemical and biological foundations of pharmacology; the gradual transformation from empirical drug discovery to rational drug design; the development of a wide variety of new drugs, as well as—more recently—the impact of molecular biology; and the re-evaluation of therapeutic strategies and priorities (Landau et al., 1999; Sneader, 2005; Church and Tansey, 2007). Thus, as products of and as agents for these advancements, drugs have become fundamental components of modern Western consumer society and of medicine and medical practice in particular.

The aims of this Research Topic were to delineate and conceptualize pharmaceutical innovation in the post-World War II era, and to highlight its roots and pathways throughout that period. Clearly, it has been impossible to include all the relevant themes, topics, and approaches that such an ambition suggests, and we have therefore selected pertinent themes of particular contemporary relevance. Participating authors have contributed to the analysis of the historical and scientific breakthroughs that have shaped pharmaceutical innovation, with an emphasis on the many journeys from rational drug discovery to biopharmaceuticals. These have included the investigation of a variety of paradigm shifting factors, such as the development and acceptance of innovative ideas; the impact of contemporary discoveries, theoretical advances, and technological developments; the influence of individual scientists; the significance of specific pioneering labs; and the varied and variable roles of industry, funding bodies, regulators, and markets.

Godfraind provides a lengthy and informative review of the discovery and development of calcium channel blockers, starting with the observation in the late nineteenth century by Sydney

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Salvatore Salomone, University of Catania, Italy

****Correspondence:***
Apostolos Zarros
azarros@outlook.com
Tilli Tansey
t.tansey@qmul.ac.uk

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Ringer that calcium was required for cardiac muscle contraction, and then progressively presenting the developments that shaped our understanding of the role of intracellular calcium in muscle contraction. From this work came studies of drugs that blocked calcium entry into cardiovascular tissues—the so-called "calcium channel blockers"—and the recognition of their therapeutic utility in a range of cardiovascular disorders. He concludes his review with a provocative suggestion, based on work on experimental animals, that calcium channel blockers may have long-term effects beyond the cardiovascular system that could be important in human pharmacotherapy.

A similar far-ranging approach is taken by Sanger and Andrews to the discovery of drugs treating nausea and vomiting; their comprehensive review not only discusses the role of serendipity underlying the relevant discoveries but also provides a critical analysis of the role of the pharmaceutical industry in both promoting and hindering drug discovery and implementation. Important issues they address are the repurposing of drugs used for the treatment of nausea and vomiting, particularly during palliative care, as well as the challenges of identifying novel anti-emetic drugs—a field in which there has not been a major breakthrough since the end of the twentieth century.

Quirke's original research article on tamoxifen as a case study in pharmaceutical innovation follows a different approach by analyzing contemporary archival records from pharmaceutical companies involved in its transition from a failed contraceptive to a best-selling chemotherapeutic for breast cancer. The history of tamoxifen as described in this paper illustrates the limits of the rational drug design, emphasizes the significance of human actors (especially Arthur Walpole of ICI), and sheds more light upon the feedback loops that exist between bench and bedside, which are stereotypical of much pharmaceutical innovation.

A different approach is taken by Al-Humadi et al. in their disease-focused review on the challenges of treating tuberculosis, with a specific emphasis on the decades between World War II and the early twenty-first century. In addition to summarizing the historical sequence of relevant drug development and use, the authors consider issues of drug resistance, drug repurposing, and problems of co-infection as factors influencing the fight against tuberculosis.

Falzone et al. consider pharmacological treatments for cancer at the beginning of the twenty-first century, against the background of a series of turning points defining modern oncology: radiotherapy; the introduction of broad-based chemotherapy; targeted therapeutics; and the recent adoption of checkpoint inhibition as a novel immunomodulatory anticancer approach. Their review emphasizes the constantly changing and evolving field of drug discovery in the fight against cancer, with a focus on the biopharmaceuticals.

A similar discussion on biopharmaceuticals is presented by Li et al. in their review of drugs for autoimmune inflammatory diseases, in which they describe the therapeutic trajectory from small-molecule compounds to anti-tumor necrosis factor (anti-TNF) biologics. In addition to analyzing the historical development of anti-inflammatory drugs after World War II, the authors consider the promise of novel TNF receptor-targeting entities that have already revolutionized the management of autoimmune diseases such as rheumatoid arthritis.

The final contribution by Gu and Pei is a shorter perspective on historical aspects of Chinese herbal medicine (CHM) ranging from traditional practice to scientific drug discovery, with a focus on the antimalarial compound artemisinin. The authors suggest that a new understanding of traditional health practices using modern systematic and *in silico* techniques such as analytical chemistry, systems biology, network pharmacology, and computational modeling could provide promising new interpretations and applications of CHM.

The articles included here cover only a small section of this huge subject of post-World War II pharmaceutical innovation. We hope that these papers will stimulate readers to delve further and reflect on the vast and varied relevant literature.

AUTHOR CONTRIBUTIONS

Both authors have contributed equally to this work.

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REVIEW
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Discovery and Development of Calcium Channel Blockers

Théophile Godfraind *

Pharmacologie, Faculté de Médecine et de Dentisterie, Université Catholique de Louvain, Bruxelles, Belgium

In the mid 1960s, experimental work on molecules under screening as coronary dilators allowed the discovery of the mechanism of calcium entry blockade by drugs later named calcium channel blockers. This paper summarizes scientific research on these small molecules interacting directly with L-type voltage-operated calcium channels. It also reports on experimental approaches translated into understanding of their therapeutic actions. The importance of calcium in muscle contraction was discovered by Sidney Ringer who reported this fact in 1883. Interest in the intracellular role of calcium arose 60 years later out of Kamada (Japan) and Heibrunn (USA) experiments in the early 1940s. Studies on pharmacology of calcium function were initiated in the mid 1960s and their therapeutic applications globally occurred in the the 1980s. The first part of this report deals with basic pharmacology in the cardiovascular system particularly in isolated arteries. In the section entitled from calcium antagonists to calcium channel blockers, it is recalled that drugs of a series of diphenylpiperazines screened *in vivo* on coronary bed precontracted by angiotensin were initially named calcium antagonists on the basis of their effect in depolarized arteries contracted by calcium. Studies on arteries contracted by catecholamines showed that the vasorelaxation resulted from blockade of calcium entry. Radiochemical and electrophysiological studies performed with dihydropyridines allowed their cellular targets to be identified with L-type voltage-operated calcium channels. The modulated receptor theory helped the understanding of their variation in affinity dependent on arterial cell membrane potential and promoted the terminology calcium channel blocker (CCB) of which the various chemical families are introduced in the paper. In the section entitled tissue selectivity of CCBs, it is shown that characteristics of the drug, properties of the tissue, and of the stimuli are important factors of their action. The high sensitivity of hypertensive animals is explained by the partial depolarization of their arteries. It is noted that they are arteriolar dilators and that they cannot be simply considered as vasodilators. The second part of this report provides key information about clinical usefulness of CCBs. A section is devoted to the controversy on their safety closed by the Allhat trial (2002). Sections are dedicated to their effect in cardiac ischemia, in cardiac arrhythmias, in atherosclerosis, in hypertension, and its complications. CCBs appear as the most commonly used for the treatment of cardiovascular diseases. As far as hypertension is concerned, globally the prevalence in adults aged 25 years and over was around 40% in 2008. Usefulness of CCBs is discussed on the basis of large clinical trials. At therapeutic dosage, they reduce the elevated blood pressure of hypertensive patients but don't change blood pressure of

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Edited by:
Tilli Tansey,
Queen Mary University of London,
United Kingdom

Reviewed by:
Siu-Lung Chan,
University of Vermont, United States
Holger Stark,
Heinrich Heine Universität Düsseldorf,
Germany
Andre Sampaio Pupo,
Universidade Estadual Paulista Júlio
Mesquita Filho, Brazil

***Correspondence:**
Théophile Godfraind
theophile.godfraind@uclouvain.be

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normotensive subjects, as was observed in animals. Those active on both L- and T-type channels are efficient in nephropathy. Alteration of cognitive function is a complication of hypertension recognized nowadays as eventually leading to dementia. This question is discussed together with the efficacy of CCBs in cognitive pathology. In the section entitled beyond the cardiovascular system, CCBs actions in migraine, neuropathic pain, and subarachnoid hemorrhage are reported. The final conclusions refer to long-term effects discovered in experimental animals that have not yet been clearly reported as being important in human pharmacotherapy.

Keywords: calcium channel blockers, voltage operated calcium channels, cardiovascular diseases, hypertension, stroke, dementia, cardiac arrhythmia

INTRODUCTION

In 1883, from a series of experiments on isolated heart, Ringer reported that calcium is required for the maintenance of cellular activity. In 1901, Stiles extended this observation to smooth muscle contraction (Ringer, 1883; Stiles, 1901). Sixty years later, Kamada in Japan (Kamada and Kinosita, 1943) and Heilbrunn in the United States (Heilbrunn and Wiercinski, 1947) discovered the role of intracellular calcium for muscle contraction. It is nowadays recognized that calcium is involved in a wide range of cellular processes being generally considered the ubiquitous second messenger.

In the 1960s, experimental work on molecules under screening for coronary dilatation allowed the discovery of the mechanism of calcium entry blockade by drugs later named as calcium channel blockers. Those drugs are now among the most commonly used agents for the treatment of cardiovascular diseases (Abernethy and Schwartz, 1999). The present paper summarizes research on small molecules interacting directly with calcium channels, also considering their therapeutic action effective in cardiovascular diseases and in neurological pathologies and their availability for medical use.

In the early sixties, many drugs have been screened either by imitation or by blockade of a typical effect of identified neurotransmitters. This was before the advent of combinatorial chemistry (Weller, 2000). The chemical structure of the neurotransmitter usually served as initial leading compound for a serial iterative processes of synthesis followed by biological assay. This serial synthesis was a rate-limiting procedure when compared to the current combinatorial chemistry allowing the preparation of many compounds in one time (Swartz, 2000). The bioassay was as simple as possible often avoiding the use of dose effect curves. Structural starting points were natural compounds, dyes or chemical entities already known for other purposes. Usually compounds obtained were termed on the basis of the lead neurotransmitter. For instance, in the group of adrenaline, they were qualified as sympathomimetics or sympatholytics. Nowadays since the deciphering of the genetic code the number of potential targets increased tremendously. In the case of G-protein coupled receptors, there are at least 800 genes representing about 4% of total human genes. Their classification is provided by NC-IUPHAR a Committee of IUPHAR, the international Union of Basic and Clinical Pharmacology.

Coming back to the early sixties, a drug discovery program was started by various pharmaceutical companies targeted coronary circulation in order to discover coronary dilators for the treatment of angina pectoris. This was initiated by Janssen Pharmaceutica with diphenylmethylpiperazines including lidoflazine, cinnarizine, flunarizine (Schaper et al., 1966), and by Knoll AG with phenylalkylamines including verapamil, D600 (Melville et al., 1964). Bayer AG followed with dihydropyridines: nifedipine, nimodipine, nisoldipine (Vater et al., 1972), and Tanabe with the benzothiazepine diltiazem (Sato et al., 1971). Later Sandoz with isradipine (PN200-110) (Hof et al., 1984), Pfizer with amlodipine (Burges et al., 1987), and others followed with other dihydropyridines (**Figure 1**).

The discovery of calcium channel blockers (CCBs) arose from pharmacological study of screened coronary dilators. This project was included in a program of pharmacological taxonomy initiated in 1964 in Godfraind's laboratory. It aimed to address qualitative and quantitative properties of drugs either under screening or already distributed for medical use. The paper published by Arunlakshana and Schild (1959) was a major basis of this program. It provided essentials of quantitative procedure allowing the definition of the action of antagonists and the estimate of their dissociation constant. Before the publication of this paper, experimental information on antagonist such as type of antagonism, potency, specificity was scarce if not inexistent. However, it must be pointed out that, in 1957, a series of papers on Drug Antagonism had been published in Pharmacological Reviews (vol. 9, Issue 2, 1 Jun 1957). Despite their great theoretical value, none of those papers reached the universal methodological usefulness of Arunlakshana and Schild's publication, which, nowadays, is receiving recognition by the name "The Schild plot" given to the house of the British Pharmacological Society in London.

FROM CALCIUM ANTAGONISTS TO CALCIUM CHANNEL BLOCKERS

The first drugs we studied were obtained from Belgian and French Pharmaceutical Companies. In Paul Jannsen's laboratory, Jagenau and Schaper had examined in dog the action of a series of diphenylmethylpiprazines on coronary arteries contracted by angiotensin (Jagenau and Schaper, 1967). Lidoflazine had been

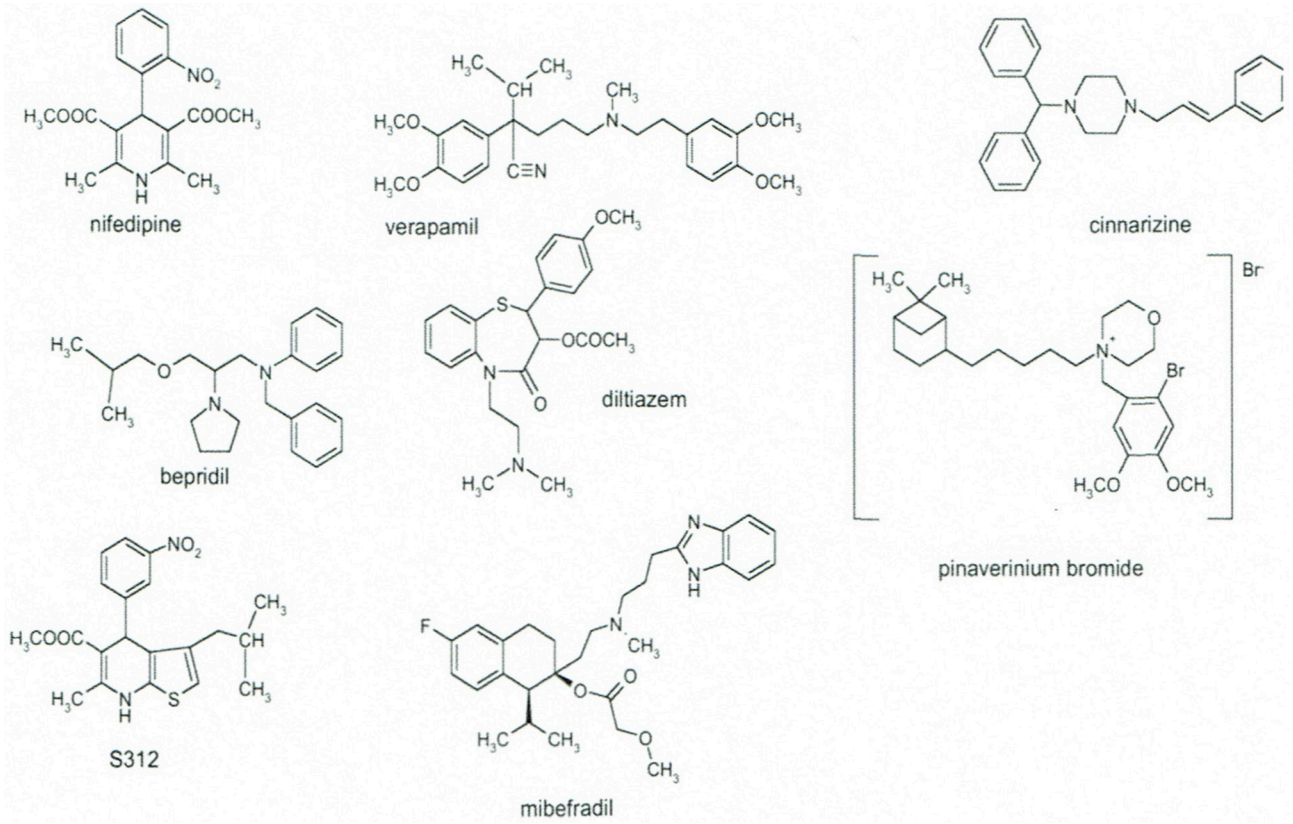

FIGURE 1 | Chemical structures of the major families of Calcium Channel Blockers as represented by their lead compounds. Modified from Godfraind (2005).

selected from this series for clinical studies in patients suffering from angina pectoris (Schaper et al., 1966). The pharmacological action of lidoflazine has been studied by Godfraind et al. on the guinea pig isolated ileum stimulated by angiotensin and other agonists (Godfraind et al., 1966). Collected estimates of pA_2 and pAh values show similarity of values of pA_2 and pAh with regard to various agonists studied. Lidoflazine, behaving as an insurmountable antagonist of similar potency for various agonists activating their specific receptors, was hypothesized to act by blocking a mechanism common to those activated receptors. The working hypothesis implied that this common mechanism involved calcium translocation. This hypothesis was based on Edman and Schild's findings that calcium is required in the bathing fluid to obtain a contraction of the Rat uterus in response to acetylcholine (Edman and Schild, 1962). On the basis of the calcium hypothesis, Godfraind and Colleagues designed experiments to examine the activity of depolarized Rat aorta contracted by 10 mM $CaCl_2$ and exposed to increasing concentrations of lidoflazine and of other drugs acting similarly. They observed that the calcium-evoked contraction was dose-dependently reduced by lidoflazine, cinnarizine, and chlorpromazine. Furthermore, the antagonist action on Ca^{2+} contraction in various arteries was overcome by increasing Ca^{2+} concentration in the perfusion fluid. On the basis of these observations Godfraind and Colleagues concluded that those drugs were acting as calcium antagonists (Godfraind et al., 1968; Godfraind and Polster, 1968). Further experiments with cinnarizine better determined the nature of this antagonism (Godfraind and Kaba, 1969a). As illustrated in **Figure 2**, calcium dose-effect curves were performed in depolarized rabbit mesenteric artery in the absence of cinnarizine and 90 min after its addition to the medium. At the lowest concentration of cinnarizine there was a displacement to the right of the calcium dose-effect curve, but at higher concentration, the antagonism was insurmountable. Such observations have been extended to other non-competitive antagonists such as chlorpromazine, papaverine, and several dihydropyridines. The dose-effect curves drawn from these experiments were similar to those obtained in agonist-antagonist studies and it supported the denomination calcium antagonist but it didn't provide indication on the mechanism of this action. Albrecht Fleckenstein and his Colleagues coincidentally made use of the term calcium antagonist in their study on the inhibitory effect of verapamil on electromechanical coupling in mammalian myocardium (Fleckenstein et al., 1969; Spedding and Paoletti, 1992).

In an attempt to better localize the action of calcium antagonists at the cellular level, the contractile response of isolated arteries to catecholamines was further examined in

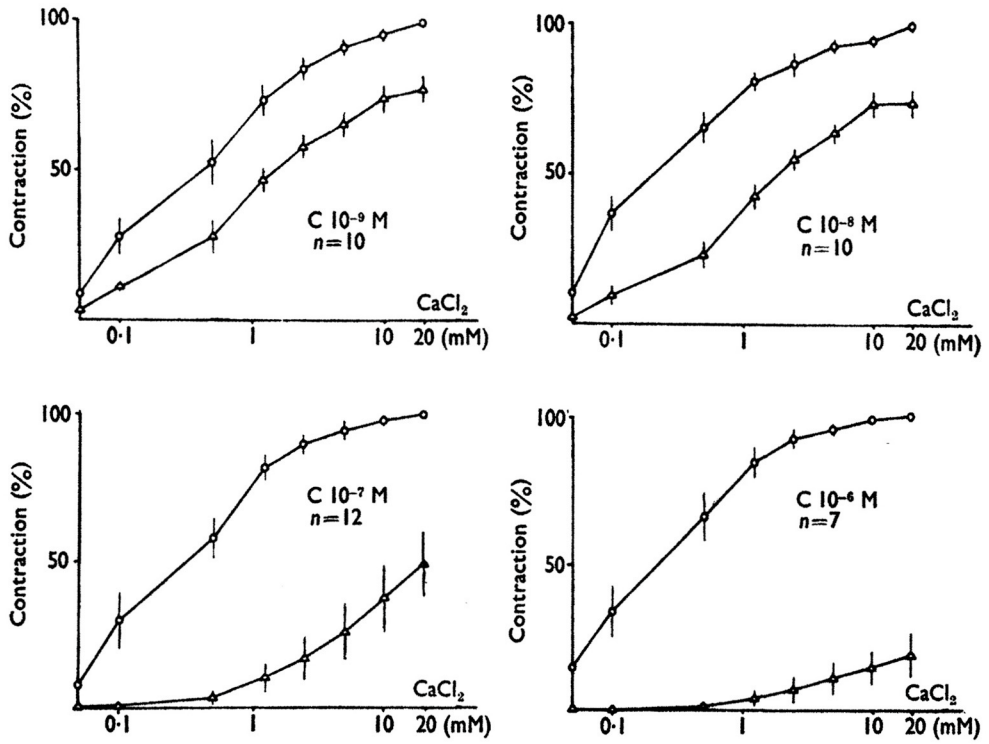

FIGURE 2 | The effect of cinnarizine on contractions evokes by Ca^{2+} in K^{+}-depolarized rabbit mesenteric arteries before and after exposure to various concentrations of cinnarizine. Responses are expressed as the percentage of maximal contraction evoked before the addition of cinnarizine. Note that the inhibitory effect of cinnarizine is observed at concentration as low as 1 nM and resembles the action of antagonists in receptor studies. This similarity suggested the terminology "calcium antagonist" (Godfraind and Kaba, 1969b; modified).

the presence of cinnarizine and of chlorpromazine. In rabbit mesenteric arteries exposed to cinnarizine 10^{-6} and 10^{-5} M, the maximum response to adrenaline recorded in Krebs solution was dose-dependently reduced down to 48 ± 3.9% of the control value with the higher dosage. The maximum response to adrenaline in Ca free solution was equal to 24.1 ± 4.4% of the maximum response in Krebs solution. It was unchanged in the presence of cinnarizine 10^{-6} and 10^{-5} M. Experimental observations were different with chlorpromazine despite its similarity of action on calcium dose-effect curve in depolarized rabbit mesenteric arteries. The maximum response to adrenaline in Krebs solution was dose-dependently depressed by chlorpromazine 10^{-7} and 10^{-6} M down to 26 ± 5.9% of the control value with the higher dosage. Responses to adrenaline in Ca free solutions were dose-dependently lowered with chlorpromazine 10^{-7} and 10^{-6} M down to 4.5 ± 1.1% with the higher dosage. Since by contrast with chlorpromazine, cinnarizine didn't alter the contraction evoked by adrenaline in calcium-free solution, it appeared unlikely that it acted as an antagonist of calcium on an intracellular target. Extracellular calcium being necessary to reach full artery contraction, a rational working hypothesis was that cinnarizine-dependent blockade of calcium entry occurred specifically at the level of cellular membrane, which was not the case for chlorpromazine that likely displayed an intracellular effect on contraction in addition to its action on calcium entry (Godfraind and Kaba, 1969a).

Studies of Ca^{2+} exchange in intact arteries have tested this hypothesis trough a comparison of dose-effect curves for inhibition of contraction and calcium fluxes in the absence and presence of calcium entry blockers. Calcium fluxes we measured with La^{3+}, which has roughly the same hydrated radius than Ca^{2+} but due to higher valence has a higher affinity than calcium for calcium binding sites. Furthermore, lanthanum doesn't enter the cell. The rate of change of the ^{45}Ca content of the tissue washed in lanthanum solution provides an estimate of the Ca fluxes across the smooth muscle cell membrane. Lanthanum was used for this purpose in various smooth muscles by several authors (Weiss and Goodman, 1969; Van Breemen et al., 1972; Karaki and Weiss, 1979). In isolated rat aorta under activation of adrenoceptors, Godfraind observed (Godfraind, 1976) a dose-dependent increase of the rate of ^{45}Ca uptake. Furthermore, in vessels loaded with ^{45}Ca solution, the rate of ^{45}Ca loss in normal solution is increased by noradrenaline. Phentolamine displaced to the right the dose effect curves of the action of noradrenaline on ^{45}Ca uptake, an effect typical of competitive antagonism with a pA_2 of 7.8, a value close to that found in contraction studies. This indicates that the activation of α-adrenoceptors is responsible for both Ca entry and contraction. Godfraind and

Dieu showed that flunarizine blocks both norepinephrine- and depolarization-dependent ^{45}Ca influx while ^{45}Ca efflux is not significantly modified (Godfraind and Dieu, 1981). The action of nifedipine is similar, but on a quantitative basis, nifedipine is more active as blocker of KCl than of norepinephrine-evoked effects. This is substantiated by IC_{50}value equal to 1.7×10^{-8} M with norepinephrine and 1.6×10^{-9} M with KCl-depolarization. Concentration inhibitory curves for Ca influx and contraction are superimposed suggesting that action on contractility is related to blockade of calcium entry through channels opened during adrenoceptors activation (Godfraind, 1983). As shown by Morel and Godfraind (1987), in the presence of dihydropyridines, the KCl-dependent contraction is reduced as a function of concentration of the blocker and of duration of the depolarization, indicating that membrane potential has an effect on dihydropyridine action. Specific binding of dihydropyridines also depends on the duration of depolarization, signifying that specific binding sites on calcium channels undergo a membrane potential dependent modification as illustrated in **Figure 3** (Morel and Godfraind, 1987). There are three main categories of calcium channels: receptor activated channels (RAC), ligand-gated channels (LGC), and voltage-operated (VOC). Briefly, VOCs are transmembrane hetero-oligomeric complexes (Catterall, 2011). A report on nomenclature (Ertel et al., 2000) and classification (Catterall et al., 2005) of voltage-operated calcium channels is provided in the IUPHAR/BPS Guide to Pharmacology accessible at http://www.guidetopharmacology.org. Calcium channel main component is the α1 subunit, which is pore-forming containing binding sites for CCBs. The 10 cloned α1 subunits are grouped into three families (**Table 1**).

The structure of those channels was initially published for channels extracted from striated muscle (Numa et al., 1990). Each α1 subunit has four repeats, each with six transmembrane domains and the pore-forming region between the two transmembrane domains S5 and S6. When inserted in lipid bilayer it exhibits properties of calcium channel, including receptor binding sites for the various CCBs. A recent structural study of bacterial Ca_V channel confirmed the different locations of the binding sites of amlodipine and verapamil. Crystallographic analysis showed that amlodipine and other dihydropyridines block the channel pore by interacting with its external, lipid-facing surface but that verapamil interacts with the intracellular side of the selectivity filter and blocks the ion-conducting pathway located in the central cavity of the pore (Tang et al., 2016). Calcium channels found in heart and vessels (L-type) are splicing products of a same gene. Isoforms are distributed unequally among the various cardiac and smooth muscles (Feron et al., 1994). Lowercase letters are used to distinguish alternatively spliced variants; $Ca_V1.2a$ corresponds to channels containing the cardiac variant and $Ca_v1.2b$ corresponds to the smooth muscle variant (Welling et al., 1997). Thus, Cav1.2 (L-type) channels are the channels operating in cardiac and smooth muscles. They are high-voltage activated and blocked by CCBs. The modulated receptor model may help to describe the interaction of CCBs with voltage-operated calcium channels. The modulated receptor theory was initially developed by Hille (1977) for local anesthetic actions on sodium channels (Godfraind, 1986). The theory proposes that the state of the channel influences the drug binding to a site located within the channel and that this state is determined by membrane potential. It has been extended to calcium channels in cardiac muscle in which blockade of calcium current by dihydropyridines calcium entry blockers is modulated by membrane potential (Lee and Tsien, 1983). Block is more pronounced when calcium current is measured during voltage clamp pulses applied from depolarizing holding potentials. In preparations that are not voltage clamped, blocking activity is influenced by cell resting potential (Sanguinetti and Kass, 1984). The model proposed by Bean for cardiac cell provides analysis of the voltage-dependence of binding of isradipine to intact arteries (Bean, 1984). As already mentioned above, K-evoked depolarization changes the affinity of CCB dihydropyridines. According to the model, the channel might present three convertible states: the resting state predominating in polarized cells where the channel is closed but available for opening, the open or activated state that is promoted by depolarization pulses beyond a certain threshold, the inactivated state unavailable for opening that is favored by prolonged depolarization. Most 1,4-dihydropyridines bind preferentially to the inactivated state of Cav1.2 channels. Therefore, depolarizing holding potentials that increase the proportion of inactivated channels, but fail to open them, enhance inhibitory potency because drug binding to inactivated channels decreases the proportion of channels that are available for activation. Assuming that K_L and K_H are dissociation constants for respectively low-affinity and high-affinity states and that, in the absence of the drug, L is the fraction of channels in the resting state, the model predicts that at any given holding potential, the concentration dependence of drug binding, and thus of calcium current inhibition, follows a simple adsorption isotherm with an apparent dissociation constant that is given by:

$$K_{app} = 1/[(L/K_L) + (1 - L)/K_H]$$

Experimental data in various organs validated this model (Bean et al., 1986; Yatani et al., 1987; Nelson and Worley, 1989; Godfraind et al., 1992a)

Such experiments allowed the pharmacological identification of CCBs with a higher affinity for inactivated than for closed channels. Those may be termed voltage-dependent CCBs. They belong to the group characterized by a high vasoselectivity. The degree of initial inhibition of a vasoconstrictor stimulus may differ along the vascular tree since the resting membrane potential varies between vessels, the apparent affinity of a voltage-dependent CCBs for Ca^{2+} channels being related to the resting membrane potential (Cauvin and van Breemen, 1985). Cerebral microvessels, which display a resting membrane potential much lower than peripheral vessels, are more sensitive to the voltage-dependent CCB nimodipine than conduit arteries. This accounts for the selectivity of nimodipine in the cerebral circulation (Morel and Godfraind, 1989). The role of voltage-dependent binding on vascular selectivity has also been observed by Sun and Triggle in a large series of dihydropyridines (Sun and Triggle, 1995).

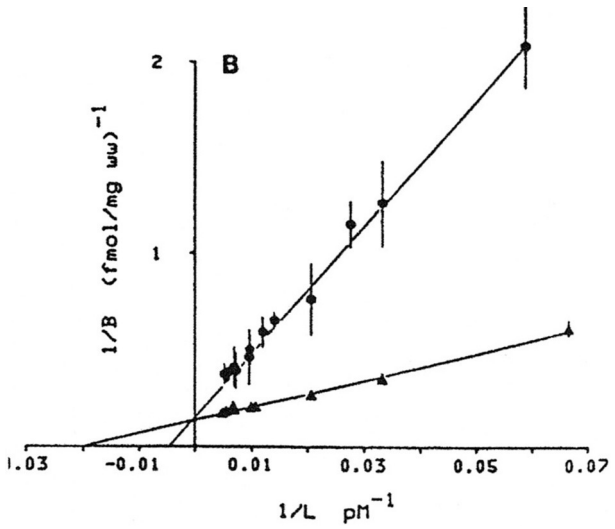

FIGURE 3 | Influence of membrane potential on isradipine binding affinity in intact artery exposed (▲) or not (●) to K^+ depolarization. Double-reciprocal plot showing change in affinity but no change of Bmax (Morel and Godfraind, 1987, 1991).

TABLE 1 | Nomenclature of voltage-operated calcium Channels (Ertel et al., 2000; Catterall et al., 2002).

Type	a_1-subunit	Splice	Current
Ca_V 1.1	α_1 1.1		L
Ca_V 1.2	α_1 1.2	Ca_V 1. 2a	L
		Ca_V 1.2b	L
		Ca_V 1.2c	L
Ca_V 1.3	α_1 1.3		L
Ca_V 1.4	α_1 1.4		L
Ca_V 2.1	α_1 2.1	Ca_V 2.1a	P/Q
		Ca_V 2.1b	
Ca_V 2.2	α_1 2.2	Ca_V 2.2a	N
		Ca_V 2.2b	N
Ca_V 2.3	α_1 2.3	Ca_V 2.3a	R
		Ca_V 2.3b	R
Ca_V 3.1	α_1 3.1		T
Ca_V 3.2	α_1 3.2		T
Ca_V 3.3	α_1 3.3		T

TISSUE SELECTIVITY OF CALCIUM CHANNEL BLOCKERS

From the previous section, it is obvious that the blocking action of CCBs on induced contraction of vessels is depending on the resting membrane potential, this action is considered selective for a given condition. Indeed the term "tissue selectivity" is used when an agent shows different degrees of potency between tissues and a preferential action in a given one. This is well-known for adrenoceptors when considering the differences between α-adrenoceptors and β-adrenoceptors for both agonists and antagonists. Several factors may be involved in tissue selectivity when considering characteristics of a given drug, properties of a given tissue and characteristics of the acting stimulus. Some examples need to be given (Godfraind et al., 1986a,b).

Characteristics of the Drug

Electrophysiological studies of various compounds considered their relative potency on L- and T-type currents. In a study with verapamil, diltiazem, lacidipine, and minefradil, it was observed that all blocked both T and L channels, but that their selectivity was differing. In this selection, mibefradil is the most selective for T-channels and lacidipine for L-channels (De Paoli et al., 2002). As shown by Furukawa et al. (1997) amlodipine has a strong blocking action on both L- and N-type calcium channels expressed in oocytes. The level of the amlodipine block on the N-type Ca^{2+} channel is similar to that on the L-type Ca^{2+} channel. The concentration dose-effect curves are nearly superimposed. IC50 values for amlodipine block on the L-type and N-type Ca^{2+} channel are 2.4 and 5.8 μM respectively at −100 mV holding potential. Action of amlodipine on the N-type Ca^{2+} channel is dependent on holding potential and extracellular pH, as observed with amlodipine block on L-type Ca^{2+} channel. Blocking action of amlodipine is enhanced by depolarized holding potential and high pH. Time course of block development by amlodipine is similar for L-type and N-type Ca^{2+} channels, but slower than the time course of block development by nifedipine for L-type Ca^{2+} channel. Amlodipine is also active on T-type currents. Bénardeau and Ertel (1998) have reported an IC_{50} value of 5.6 μM for T- channel block in guinea-pig atria. It is of note that in this concentration range, amlodipine is a powerful ACE inhibitor, which through the preservation of bradykinine stimulates the release of NO from endothelial cells (Xu et al., 2002). This latter effect is important in view of the synergism for vascular relaxation existing between NO and CCBs (Godfraind and Salomone, 1996; Salomone et al., 1996). Those various properties of amlodipine need to be taken into account when examining the therapeutic mode of action of this drug. Edward Perez-Reyes et al have studied several CCBs on recombinant $Ca_V3.2$ channels (Perez-Reyes et al., 2009). They noted that four clinically approved antihypertensive drugs (efonidipine, felodipine, isradipine, and nitrendipine) are potent T-channel blockers ($IC_{(50)} < 3$ microM). However, highly prescribed dihydropyridines, such as amlodipine and nifedipine, are 10-fold less potent on T-channels than on L-channels, these are more appropriate for use in research studies on blockade of L-type currents in therapy. Cilnidipine is highly potent against N-type current (Uneyama et al., 1997). It may be anticipated that those CCBs acting at the level of N-type currents and thereby impairing catecholamines release from nerve endings, should blunt the sympathetic reflex following vasodilatation. This is the basis of another difference of selectivity between drugs. Therefore, it is appropriate to consider the selectivity window, which can be related not only to the ratio of active concentrations blocking L-type channels and other channels (T or N-type) but also to the ratio of active concentrations blocking L-type channels and other membrane processes (such as receptors, other channels, or transporters). For instance D600 blocks not only

L-type channels but also the α-adrenoceptors (Godfraind et al., 1992b). Dihydropyridines may also block Na channels, but at higher concentration than calcium channels (Yatani et al., 1988). Another example is flunarizine, which does not only interfere with the various voltage-operated calcium channels, but which is also acting on the release of dopamine by nerve terminals (Terland and Flatmark, 1999). Great attention has been devoted to the selectivity for other ion channels, in particular K-channels, verapamil has the lowest ratio CaCh/KCh of the drugs tested. It is acting at nearly the same concentration on both channels; the consequences of this property have not yet been evaluated (Grace and Camm, 2000; Hatano et al., 2003). Interestingly, the two splice types of α_1 subunit have different sensitivity for nisoldipine a dihydropyridine (DHP) Ca^{2+} channel blocker with a high vascular selectivity (Godfraind et al., 1992b). Nisoldipine is a more powerful blocker of inward current in cells transfected with $\alpha_1$1.2b isoform cDNA than in those transfected with $\alpha_1$1.2a isoform cDNA. Nicole Morel et al have examined if this property is shared by other DHP and non-DHP Ca^{2+} channel blockers (Morel et al., 1998). They used Chinese hamster ovary cells (CHO) transfected either with cDNA encoding for the $\alpha_1$1.2a or with cDNA encoding for the $\alpha_1$1.2b subunit of the L-type Ca^{2+} channel, issuing respectively from rabbit heart and lung smooth muscle (Welling et al., 1993). The Ca^{2+} channel blocking activity of three neutral DHP derivatives, (+)-PN 200-110, nifedipine and nisoldipine, which show different degrees of vascular selectivity (Godfraind et al., 1992a,b), and one positively charged derivative SDZ 207-180 (Kass et al., 1991) was compared to that of the phenylalkylamine verapamil, which is equipotent in cardiac and vascular tissue (see below) and to that of pinaverium bromide, a non-DHP compound with a quaternary ammonium, reported to show intestinal selectivity (Christen, 1990). The voltage-dependent current mediated by the α1 subunit of the L-type Ca^{2+} channel ($I\alpha_1$) was recorded with the whole-cell configuration of the patch-clamp technique using barium ions as charge carrier. Binding affinity of Ca^{2+} channel blockers was also assessed in displacement studies using the Ca^{2+} channel ligand [^{3}H]-(+)-PN 200-110. Experimental results show that neutral dihydropyridines (nifedipine, nisoldipine, (+)-PN200-110) were more potent inhibitors of $\alpha_1$1.2b subunit than of $\alpha_1$1.2a subunit. This difference was more marked at a holding potential of −100 mV than at −50 mV. SDZ 207-180 (an ionized dihydropyridine) exhibited the same potency on the two isoforms. Pinaverium (ionized non-dihydropyridine derivative) was 2-and 4-fold more potent on $\alpha_1$1.2a than on $\alpha_1$1.2b subunit at Vh of −100 mV and −50 mV, respectively. At both voltages, the two isoforms were equally sensitive to verapamil. Neutral dihydropyridines had a higher affinity for the $\alpha_1$1.2b than for the $\alpha_1$1.2a subunit as shown by binding experiments with [^{3}H]-(+)-PN 200-110. SDZ 207-180 had the same affnity for the two isoforms and pinaverium had a higher affinity for the $\alpha_1$1.2a subunit than for the $\alpha_1$1.2b subunit.

All these results show that marked differences are observable among Ca^{2+} channel blockers concerning their selectivity not only for the three subfamilies of calcium channels but also for the $\alpha_1$1.2a and $\alpha_1$1.2b subunits. Therefore, this justifies studies on the pharmacological profile of drugs belonging to a same chemical family in order to see if they present or not uniformity in action.

Properties of the Tissue and Characteristics of the Stimuli

In order to characterize these factors, we may focus on some characteristics of the selectivity of CCBs in the cardiovascular system. They were identified in experiments with arteries and myocardium isolated in different species. In view of species-dependent selectivity, data obtained with human preparations are important for translational Medicine. Indeed, potency ratios of nifedipine expressed as ratio of IC_{50} for cardiac inotropism over IC_{50} for contraction of depolarized vessel are dissimilar within species, the highest value being obtained in rat and the lowest in guinea pig. Comparing various CCBs in guinea-pig, Spedding et al. (1990) showed that the ratio of IC50 values heart/vessels is 0.3 for diltiazem, 1.3 for verapamil and 3.1 for nifedipine. Experiments from Triggle's laboratory are in complete agreement with the results just mentioned (Triggle, 1999).

^{3}H(+)-isradipine, was used in order to estimate the apparent affinity of dihydropyridine-binding sites in plasma membranes of human coronary artery and human myocardium. The sequence of affinity was nisoldipine > isradipine > nifedipine. The dissociation constant values may be compared to functional values of IC_{50} obtained in other experiments (Godfraind et al., 1992b). Functional estimates of IC_{50} of CCBs in human coronary arteries exposed to serotonin are close to radioligand estimates of the apparent dissociation constant in corresponding plasma membranes. In human heart, functional IC_{50} values are much higher than radiochemical K_i (or K_d) values estimated in plasma membranes. There is a 10,000-fold difference for nisoldipine, a 1,298-fold difference for isradipine and a 27-fold difference for nifedipine. Nisoldipine is much less active than nifedipine in cardiac preparation and much more active in human coronary artery. Differences between CCBs are illustrated in **Figure 4**, which illustrates log scale of the reciprocal of the concentration needed to block 50% of the contractile activity (IC_{50}) in human coronary artery, human internal mammary artery, and human myocardium. It shows that diltiazem and verapamil have the same potency in arteries and in myocardium. For nisoldipine, the potency sequence is coronary artery >> mammary artery >> myocardium, whereas for nifedipine, the sequence is coronary artery = mammary artery >>myocardium (Godfraind et al., 1992b). Other experiments (Sarsero et al., 1998), using preparations from human atria and aortic vasa vasorum, confirm the vasoselectivity of nisoldipine, and the cardioselectivity of verapamil. Several factors might be involved in the difference in sensitivity between heart and vessels. In cardiac myocytes, sarcolemmal Ca channels bring Ca into the cell (L- and T-type Ca channels). This Ca influx contributes an inward current, which makes (or keeps) the membrane potential more positive and activates contraction, being second messenger in the excitation-contraction (E-C) coupling. It activates intracellular Ca channels allowing the release of Ca from the sarcoplasmic reticulum (SR) and endoplasmic reticulum (ER; ryanodine and IP_3 receptors), which amplifies the function of Ca that enters via the sarcolemma

(Bers and Perez-Reyes, 1999). The ryanodine receptors (RyR) are more numerous that the L-type channels in the sarcolemma. According to Wibo et al. (1991), the ratio is 1 over 9.

The cardiac E-C mechanism is influenced in different circumstances. These include autonomic modulation, L-arginine-NO pathway activation and pathological conditions related or not to hypertension and atherosclerosis (Balligand et al., 1993; Maier and Bers, 2002). The classical long plateau of cardiac action potentials is essential for preventing re-excitation and arrhythmias. As already pointed out above, the α_1-subunit is one among the five subunits constitutive of voltage-operated calcium channels. When inserted in lipid bilayers, it shows properties of calcium channels particularly the binding sites for the various CCBs. Different genes are coding the various calcium channels types. Nevertheless, splicing products of the same gene such as l-type calcium channels found in heart and vessels have different affinities for dihydropyridines, but not for verapamil. This observation is consistent with the absence of vascular selectivity of verapamil (see above). However, differences in sensitivities between heart and vessels observed in pharmacological experiments don't appear to be only due to affinity ratio and other factors need to be considered (Morel et al., 1998). Feron et al. (1994) have reported tissue-dependent developmental regulation. During development, there is a profound modification of the localization of L-type Ca channels from the peripheral plasma membrane to the junctional structures (**Figure 5**) (Wibo et al., 1991). The functional consequence results in a lower sensitivity to CCBs of adult over neonatal hearts (Wibo et al., 1991).

Time-course experiments may provide additional information on the tissue selectivity. The inhibitory action of nisoldipine and other dihydropyridines on contractions is characterized by a marked time-dependency following initiation of the depolarizing stimulus in pre-incubated arteries: the inhibition increases slowly after depolarization to attain a steady-state value. By contrast, in the presence of verapamil and diltiazem, the inhibitory action is immediate, and sustained for the duration of the depolarization. Inhibition by dihydropyridines follows the kinetics of their binding to receptors connected to different modulations of calcium channels due to variable durations of the stimuli. The rate of association in intact depolarized tissues follows a pseudo-first-order kinetics similar to the association rate constant for purified calcium channels (Wibo et al., 1988). The short systolic depolarization ($\pm$0.4 s) does not favor a proportion of inactivated calcium channels similar to that obtained after the longer stimulus duration ($\pm$6 min) required to full activation of vascular smooth muscle. This controls the concentration of nisoldipine required to occupy a given proportion of inactivated channels for a short systolic depolarization or for a time required to fully activate a smooth muscle. For an occupation of 50 per cent of receptor sites at the end of the stimulus, the ratio of concentrations in cardiac vs. arterial muscle is equal to 1,500, a value similar to that found in contraction studies (Godfraind et al., 1987). *In vivo* studies are fully consistent with *in vitro* studies (Rousseau et al., 1994).

The degree of inhibition of response to a given vasoconstrictor may be different between arteries exposed to the same CCB.

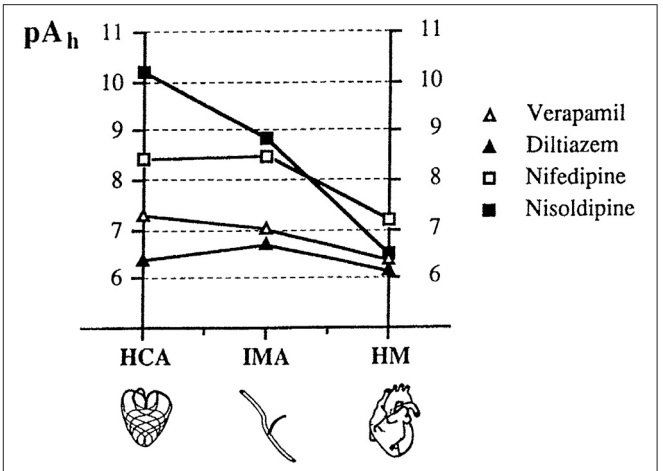

FIGURE 4 | Logarithmic scale of the IC_{50} (pA_h) of CCBs in human coronary (HCA) and internal mammary (IMA) arteries stimulated by serotonin and in electrically stimulated human myocardium (HM). IC_{50} is the concentration producing 50% reduction of the contraction. Modified from Godfraind et al. (1992b).

The curves relating inhibition by nisoldipine of serotonin-evoked tonic contraction in various arteries are not superimposed. For instance inhibition of the tonic contraction to serotonin is greater in human coronary artery than in human internal mammary artery. This extends earlier reports on inhibition of contractile responses depending on the type of vessel: resistance arteries being more inhibited than conduit arteries (Godfraind and Polster, 1968; Godfraind et al., 1968). Another illustration of heterogeneity is related to the mode of activation of the contraction by various adrenoceptor agonists. The maximum contractions of rat aorta evoked by α_1-agonists noradrenaline or phenylephrine are less inhibited than maximum contractions evoked by α_2 agonists such as clonidine and oxymethazoline. Such observations have been extended to various CCBs and to various vessels. For instance, the maximal contraction evoked by ET-1 in human isolated coronary arteries exposed or not to nisoldipine (1 μM) (Balligand and Godfraind, 1994) is inhibited by 51% in distal arteries whereas it is inhibited by only 35% in proximal segments. They emphasize the hypothesis that vascular heterogeneity may be, at least partly, related to the proportion of contractile responses resistant or not to calcium-channel blockade (Godfraind, 1994). It is likely that the interaction of agonists with their receptors activates targets other than voltage-operated calcium channels such as protein kinase C, thus resulting in an increase in the contractile proteins sensitivity to calcium (Karaki, 1989; Nishimura et al., 1990). The importance of this mechanism that is independent of Ca entry is likely to vary between different smooth muscles and to play a role in vascular selectivity. Among factors responsible for heterogeneity among vessels, not only the specificity of the blocker and its voltage-dependency but also its tissue pharmacokinetics needs to be taken into account. For instance, Angelico et al. (1999) have observed in isolated rabbit aorta stimulated by high KCl that 50% relaxation was reached at different times according to the CCB tested. At 10

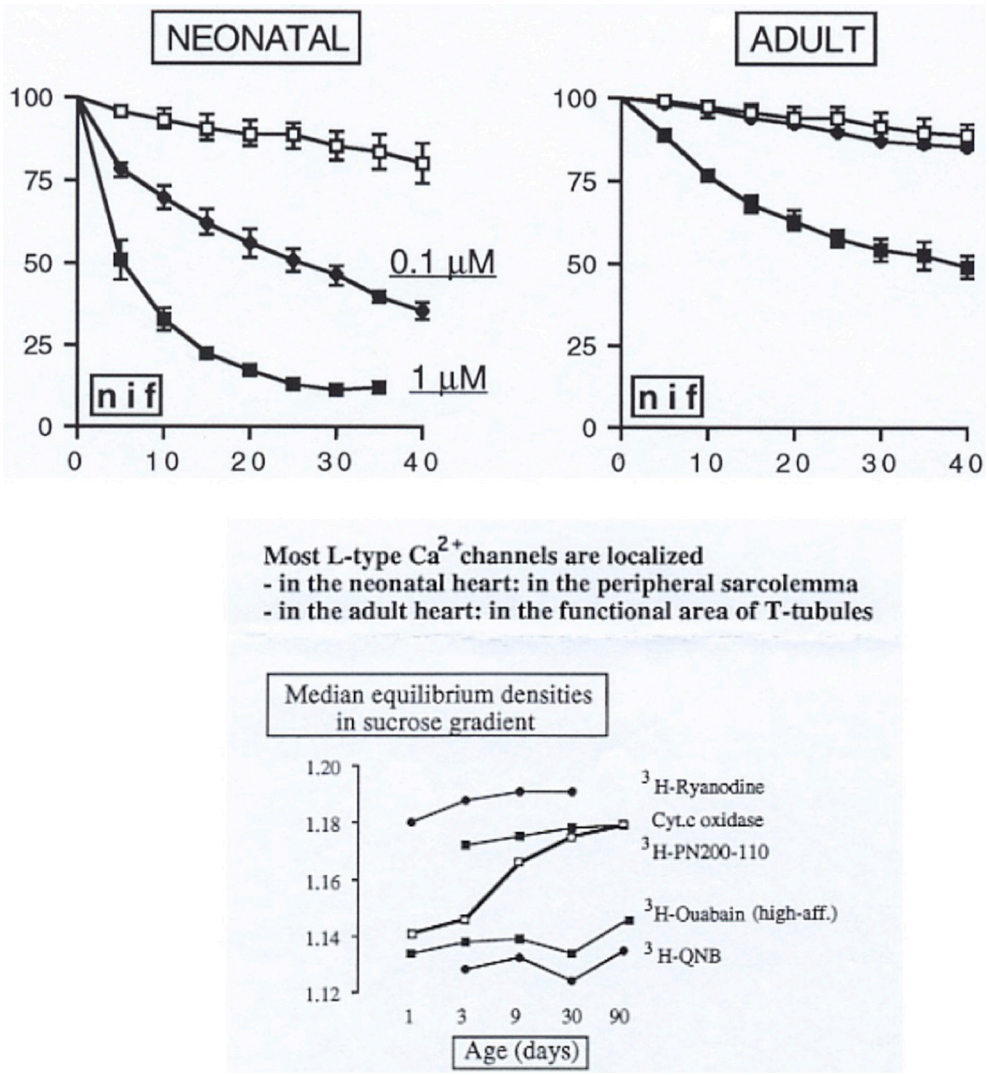

FIGURE 5 | Upper graphs show different sensitivity to negative inotropic action of nifedipine in neonatal and adult heart. Lower graph shows cellular location change of CCB as a function of age in rat heart. Modified from Wibo et al. (1991).

nM concentration 50% relaxation was reached after 210 min with lercanidipine, 278 min with amlodipine, 135 min with lacidipine, 75 min with nitrendipine, and 70 min with felodipine. On the other hand, when studying the heart ventricle, the rate sequence was lacidipine > amlodipine > felodipine > lercanidipine = nitrendipine, indicating that comparison between drugs requires appropriate experimental conditions in order to validate the IC_{50} ratio. It is noticeable that arteries and veins display different sensitivities to CCBs and to various vasoconstrictors. This has been documented with endothelin (ET1) in rings of canine vessels (Miller et al., 1989) and in isolated human vessels. In the latter, the contractile response of coronary artery to ET1 is blocked by CCBs by contrast the response of coronary vein is insensitive (Balligand and Godfraind, 1994). This differential sensitivity is also observable with noradrenaline (Sjoberg et al., 1987) as well as on arteries and veins in humans (Robinson et al., 1980).

ACUTE HEMODYNAMIC EFFECTS OF CCBS

Systemic Hemodynamic

Calcium channel blockers are used in therapy for long-term even life-long-treatment, nevertheless it is of interest to examine the acute hemodynamic changes evoked when those agents are administered at pharmacologically active doses. Such a study allows comparing the various CCBs from a functional point of view considering potency, pharmacokinetics and tissue selectivity. The hemodynamic action of CCBs has been studied *in vivo* with most of the compounds, once they were identified *in vitro*. Due to the complexity of the CCBs-evoked hemodynamic reflexes and to their tissue selectivity, those drugs exhibit variations in action according to the vascular bed or the species so far considered particularly at the level of coronary, renal, and cerebral circulations. In conscious rats, Ishii et al. (1980) have

observed that nifedipine decreased blood pressure at doses much lower in SHR than in WKY. Knorr and Garthoff (1984) compared nitrendipine and hydralazine in SHR and WKY. They reported that the vasodilator hydralazine is equipotent in both strains at variance with nitrendipine less active on blood pressure in WKY than in SHR. Consistent with hemodynamic observations (Kazda and Knorr, 1990), these studies show that the activity profile of CCBs in cardiovascular system is different from the activity profile of classical arteriolar vasodilators. Furthermore, during chronic administration, the decrease of blood pressure occurs without change of the cardiac frequency (Frohlich, 1986).

Following the administration of increasing intravenous doses of nitrendipine. Taylor et al. (1984) there is a progressive reduction of blood pressure, reaching a maximum effect at 50% of initial value, accompanied by a reduced resistance and an increase of blood flow in the femoral and mesenteric territories. Taylor and Colleagues showed that CCBs are arteriolar dilatators with differing activities according to the circulation bed with greatest effect in the coronary bed. It is important to note that no significant hemodynamic effects have been observed in the venous circulation. In dog the coronary circulation is more influenced that the peripheral one. Acute response to IV administration evokes major sympathetic reflexes characterized by an increased cardiac rhythm. In human after sublingual nifedipine, the vasodilator action does occur mainly on the arterial side with a modest effect on the venous side (Merillon et al., 1980). It is worth mentioning that ACE inhibitors dilate to the same extent the arterial and the venous beds. Variations in regional sensitivity to the vasodilatation effects of CCBs are in agreement with *in vitro* vascular selectivity. Following the injection of nifedipine there is a large increase in coronary flow with reduced myocardial oxygen consumption. Dilatation of resistance vessels in the coronary bed overcomes reflex coronary vasoconstriction occurring physiologically when myocardial oxygen consumption is decreasing (Berdeaux et al., 1990).

Mesenteric and Renal Beds

Janssen et al. (2001) have studied in rats the regional hemodynamic effects of long acting CCBs barnidipine and amlodipine. In male adult SHRs, Doppler flow probes and catheters allowed the measure of renal (RVR), mesenteric (MVR), and hindquarter (HQVR) vascular resistance changes. One week after surgery, barnidipine, or amlodipine were intravenously administered at three doses causing comparable reductions in mean arterial pressure (MAP). Barnidipine at doses of 3, 10, and 30 μg/kg reduced MAP (± SEM) by 8 ± 2, 26 ± 3, and 45 ± 4 mmHg ($n = 10$). Amlodipine achieved similar effects on MAP at doses of 100, 300, and 1,000 μg/kg. Barnidipine at 3 and 10 μg/kg reduced MVR (% ± SEM) by 4 ± 4 and 19 ± 4, and RVR by 8 ± 2 and 15 ± 4, respectively. In contrast, HQVR remained unaltered. Similar observations were done with amlodipine, except that changes in RVR were half of those found after barnidipine. By contrast short-acting nifedipine and isradipine reduced HQVR and not RVR.

Cerebral Blood Flow

Change in blood flow resulting from increase in cardiac output has consequences in tissue perfusion according to the vascular bed. Physical exercise producing a 3-fold increase in cardiac output leads to a 10-fold increase in blood flow to skeletal muscle, halves renal blood flow, but does not modify cerebral blood flow (Wade and Bishop, 1962). Antihypertensive drugs can have pronounced effects on CBF. As discussed by Atkinson and Capdeville (1990), those agents can be divided into three categories. In the first are found those that decrease CBF, which can further be subdivided into drugs which interfere with noradrenergic vascular tone such as ganglion blocking agents, centrally acting hypertensive drugs like clonidine, alpha adrenoreceptor blocking agents such as prazosin and various vasodilators such as diazoxide, sodium nitroprusside and nitroglycerin. A second group is composed of drugs that have minor effects on CBF. They include antagonists of the vascular 5-HT2 receptor such as ketanserin, that markedly increase cardiac output but produce very little change in CBF, blood flow being distributed to the gut, kidneys and skeletal muscles. Likewise beta-blockers and ACEI have no significant effect on CBF. A third category of antihypertensive drugs is composed of those that increase CBF, including CCBs. The increase in cardiac output produced by CCBs, such as felodipine, for example, is accompanied by a marked increase in CBF, similar to that observed in coronary blood flow, and far above that seen in other organs such as the kidney, the gut, and the skeletal muscles (Bolt and Saxena, 1984). This pharmacological profile is shared with other vasodilators such as potassium channel activators and hydralazine, but the cerebrovascular dilator effect of dihydropyridine CCBs is the most pronounced.

Hara et al. (1999) have studied in anesthetized rats the effects of lomerizine, a CCB of the piperazine group, on cerebral blood flow by laser Doppler flowmetry and on vertebral blood flow in anesthetized beagle dogs with an electromagnetic flowmeter. They observed that lomerizine (2.5 and 5 mg/kg, intraduodenally) dose-dependently increased vertebral blood flow in dogs without significantly changing BP or HR. With 10-mg/kg intraduodenal lomerazine, vertebral blood flow remained elevated from 20 to 240 min after administration despite a concomitant decrease of BP occurring from 20 to 120 min. This confirms the concept reported above that CCBs might oppose the vasoconstrictive reflex in cerebral vessels, which is physiologically activated in response to a fall in blood pressure.

Regional Response and Differences between CCBs

Studies on anesthetized dogs help to characterize differences between molecules, taking into account their potency, the time course of their effect and their specificity of action on a given vascular bed. In view of renal hemodynamic, actions on renal blood flow, and on natriuresis provide the opportunity to

clarify the mechanisms involved in the therapy of hypertension. Calcium channel blockers do not cause sodium retention, an undesired effect that is observed with vasodilators including α-blockers, hydralazine, and minoxidil. By contrast, CCBs increase sodium excretion when administered acutely to hypertensive humans and animals (Kazda and Knorr, 1990). Mechanisms involved in the natriuresis evoked by CCBs are associated with various processes such as changes in renal hemodynamic, direct effects on renal tubules or indirect through regulation of vasoactive substances. Several lines of evidence suggest that they possess multiple actions that could be independent of calcium channel blockade. CCBs increase nitric oxide (NO) production both *in vitro* and *in vivo* (Krenek et al., 2001). It is known that NO induces natriuresis by inhibiting sodium reabsorption by the nephron. Another possible mechanism is blockade of T-type channels involved in the renal function. Nifedipine inhibits L-type calcium channels at concentrations much lower than T-type channels, but some other CCBs such as efonidipine block at similar concentration T-type and L-type calcium channels. T-type calcium channels have been identified in vascular smooth muscle cells that are involved in renal blood flow (Godfraind, 1994). In renal tissue, L-type calcium channels are found only in the afferent arterioles, while N-type and T-type calcium channels are located in both efferent and afferent arterioles. Therefore, CCBs that block either T-type or N-type calcium channels may exert renoprotective effects through dilatation of the efferent artery; this avoids hyperfiltration injury of the glomerulus. It has been established that T-type CCBs exert a renal protective action by ameliorating glomerular microcirculation via vasodilator activity on both afferent and efferent arterioles. Additionally, blockade of the T-type Ca channel suppresses inflammatory processes, renin-angiotensin-aldosterone system activation, and oxidative stress. Honda et al. (2001) have compared compounds with different actions on renal afferent and efferent arterioles and have examined whether these CCBs exert divergent actions on natriuresis. Their effect on renal arterioles obviously depends on the molecule so far examined. Intravenous infusion of nifedipine (L-type blocker), efonidipine (L/T-type blocker), or mibefradil (predominant T-type blocker) into anesthetized dogs elicits similar, albeit modest, reductions in blood pressure. The CCB-dependent urinary nitrate/nitrite excretion is dose-dependent but it shows no differences between the various CCBs, indicating that they have similar action on NO production and that this action could not account for the differences observed between drugs in their renal hemodynamic and natriuretic actions. It is proposed that the inhibition of tubular sodium reabsorption associated with the increased post-glomerular blood flow are involved in the natriuretic action of CCBs.

Thus in various situations, hemodynamic studies point to major differences between CCBs, not only in potency but also in selectivity of action. This emphasizes the observation that even drugs having a similar specific molecular target may behave differently *in vivo*, because of the uneven distribution of this target among organs, which become important sites of action in some pathophysiological situations.

KEY INFORMATION ABOUT CLINICAL USEFULNESS OF CCBS

The Therapeutic Indications of CCBs

The most common indications of CCBs are hypertension and other major cardiovascular disorders. Because vascular risk factors such as hypertension, obesity, and diabetes have been considered as potentially modifiable risk factors for Alzheimer disease, as well as vascular dementia (Barnes and Yaffe, 2011), management of cognitive disorders are included under the same list of indications. A second list comprises neurological disturbances.

In the United States, eight of the major CCBs are currently marketed. Their indications and adverse effects depending on the specific drug are summarized on **Table 2**.

In addition, the dihydropyridine Clevidipine an ultra-short acting drug is approved by the FDA for perioperative use as injectable emulsion in case of severe hypertension in cardiac or non-cardiac intervention. The half-live is of about 2 min due to esterase hydrolysis. The drug is marketed under the name Cleviprex. In Belgium, in addition to verapamil and diltiazem, 11 dihydropyridines are marketed, including those listed in **Table 2**: Barnidipine (Vasexten), Lacidipine (Motens), Lercanidipine (Lercanimylan, Zanidip), Nimodipine (Nimotop), Nitredipine (Baypress).

Treatments accepted globally with CCBs comprise stable angina and include supraventricular arrhythmias treated with non-dihydropyridine CCBs, but exclude systolic dysfunction. As far as management of hypertension is concerned, the medical community at large is reaching a consensus based on evidence in recommending CCBs in initial treatment of hypertension as reported in major guidelines such as JNC 8 (James et al., 2014) and NICE Clinical Guidelines 127 (http://www.nice.org.uk/CG127) that approves combination with a diuretic in patients with diabetes. An algorithm support of treatment is provided but isn't imposed on physician best clinical judgment for adult patients and for black people of any age (Go et al., 2014).

Meta-analysis comparing clinical effectiveness within dihydropyridine-type CCBs did not indicate major differences (McDonagh et al., 2005). However, based on home BP monitoring, a crossover study of amlodipine vs. nifedipine showed that amlodipine had a lower antihypertensive effect with a lesser pulse rate during the critical morning period (Ryuzaki et al., 2007). More head-to-head studies are needed since pharmacological differences have been observed in the profile of the various dihydropyridines as reported in this paper from experimental observations. This is important when considering validity of translating pharmacological data to patient's treatment. There is a current tendency for the prescription of combinations of antihypertensive drugs. This question has been discussed in another recent publication (Godfraind, 2014).

The Controversy on the Safety of CCBs

In the early 1990s questions on the safety of CCBs raised when lidoflazine that had been considered as a very promising

TABLE 2 | Calcium Channel Blockers currently marketed in the United States.

Drug	Proprietary name	Indications, USA
Amlodipine	Norvasc	Hypertension; Chronic, stable, and vasospastic angina
Diltiazem	Tiazac; Cardizem; Cartia; Dilacor	Hypertension; chronic, stable, and vasospastic angina; atrial fibrillation or flutter; paroxysmal supraventricular tachycardia
Felodipine	Plendil	Hypertension
Isradipine	Dynacirc	Hypertension
Nicardipine	Cardene	Hypertension; angina
Nifedipine	Adalat; Procardia	Hypertension; angina
Nisoldipine	Sular	Hypertension
Verapamil	Calan; Covera; Verelan	Hypertension Angina Atrial fibrillation Or flutter Paroxysmal supraventricular Tachycardia

therapeutic agent (Jenkins et al., 1981) was withdrawn after publication that its effects were both beneficial and detrimental. The main observation reported is summarized in the following sentences: "During the randomized, placebo-controlled phase of the study with 7-week treatment periods, 9 of 11 patients who completed this phase of the study preferred lidoflazine and all demonstrated improved exercise capacity with lidoflazine compared to placebo. However, three patients developed malignant ventricular arrhythmias, and 1 died while taking lidoflazine, resulting in termination of the study" (Cannon et al., 1990). Arrhythmias have been associated with QT prolongation (Ridley et al., 2004). Therefore, a major question was whether or not such observations were related to class effects or just to a specific chemical structure. Analysis of the literature on CCBs was a rational approach to this question. Meta-analysis of clinical trials of nifedipine was published in 1995 (Furberg et al., 1995). Conclusion of this publication was that moderate to high doses of the short-acting nifedipine increased mortality in patients with coronary artery disease (CHD). This conclusion was refuted by other authors (Opie and Messerli, 1995; Opie et al., 2000; Opie and Schall, 2002) who didn't nevertheless recommend fast administration of nifedipine because of the occurrence of the cardiovascular reflex to acute hypotension. The controversy ended after the publication of ALLHAT a large antihypertensive trial sponsored by the National Heart, Lung, and Blood Institute (Group TAOaCftACR, 2002; Chrysant, 2003). In more than 30,000 high-risk patients with hypertension, this trial compared amlodipine (CCB), lisinopril (ACEI), and chlorthalidone (diuretic), respectively on CHD. No differences occurred in primary end point (combination of fatal CHD and acute myocardial infarction). A large series of analytical and commentary papers followed the publication, highlighting the importance of these findings for the management of patients with hypertension (Leenen et al., 2006). ACTION trial, which studied clinical outcomes in 7,665 patients of 63.5 years mean age, extended to nifedipine GITS the safety conclusions obtained from ALLHAT (Poole-Wilson et al., 2004). Nowadays the controversy on safety of CCBs is closed.

The Action of Calcium Channel Blockers in Cardiac Ischemia

Early Clinical Studies of CCBs in Ischemic Heart Disease

Ischemic Heart Disease affects the supply of blood to the heart. Blood vessels might be blocked due to deposition of cholesterol in their walls. This reduces the supply of oxygen and nutrients to the heart muscles. This may eventually lead to destruction of an area of heart tissue, inducing a heart attack. Ischemic heart disease is the most common cause of death in many countries around the world. Causal factors including hypertension have been listed above. The clinical aspects of ischemic heart disease are usually expressed by Angina Pectoris, an acute chest pain attributed to chronic stable effort angina, to vasospastic angina, to unstable angina, and acute myocardial infarction. Heart failure might follow a resulting weakness of the heart muscle.

Chronic Stable Effort Angina

In the 1970s, about one century after the introduction of nitroglycerine by William Murrel (1853–1912) in the management of angina pectoris (Smith and Hart, 1971), it was reported that agents other than nitrates treat efficiently this pathology. β-Blockers have preceded CCBs, therefore several trials have been designed comparing CCBs with propranolol as well as over placebo.

The action of nifedipine in stable effort angina

Nifedipine action has been widely investigated in patients suffering of stable angina. Here are briefly reported earliest studies that established the therapeutic action of nifedipine in angina pectoris. Its action over placebo appeared to be highly significant (Terry, 1982; Sherman and Liang, 1983). Exercise-induced ST segment depression was reduced after 20 mg nifedipine (sublingual) (Hopf et al., 1983). This effect was also observed after oral administration and it was dose-related (Hopf et al., 1983). This beneficial response was also evoked after intravenous and intracoronary administration. When given orally at 20 mg three times per day, nifedipine action persisted during prolonged studies. When nifedipine and nitroglycerin were given sublingually, the authors have estimated the following parameters: work load, maximum heart rate, blood pressure at rest while seated on bicycle, maximum systolic blood pressure measured by indirect measurement using a mercury column manometer, maximum rate pressure product, ST-segment depression at controlled heart rate. Results showed clearly that nifedipine increased total work and decreased the depression of the ST-segment. Nifedipine was also compared to β-blockers (Lynch et al., 1980; Dargie et al., 1981). Effects of nifedipine (60–90 mg per day) monotherapy and propranolol (240 mg per day) monotherapy on symptoms, angina threshold, and cardiac function in patients with chronic stable angina

were studied in a placebo-controlled double-blind crossover study. After a 2-week placebo period, patients were randomly ascribed to receive either nifedipine or propranolol for a 5-week treatment period, after which they crossed over to the alternative regimen. All 21 patients were men with chronic stable angina pectoris, 13 of whom had symptoms both at rest and on exertion. In patients taking either nifedipine or propranolol, New York Heart Association functional class improved and nitroglycerin consumption decreased. Nifedipine and propranolol were equally effective in relieving exertion ischemia. Exercise wall motion also improved with both drugs. Propranolol treatment decreased exercise cardiac output by 14 percent ($p = 0.01$) through its effect on heart rate. Nifedipine had the advantage of preserving cardiac output during exercise (Higginbotham et al., 1989). The antianginal effects of nifedipine and propranolol (alone or in combination) compared with placebo were examined in a double-blind clinical trial that included 16 patients with chronic stable angina triggered by effort. Each of the active drugs significantly reduced frequency of chest pain and nitroglycerin consumption. The combination of nifedipine and propranolol increased significantly the effectiveness. About 60 percent of all episodes of ST segment depression were painless and responded to therapy as did episodes associated with chest pain (Dargie et al., 1981). Studies realized in the 1990s and later reinforced evidence of the anti-ischemic effect of β-blockers and CCBs by showing that dihydropyridine-CCBs and β-adrenergic blocking agents are similarly effective in effort agina associated with hypertension (Pfisterer et al., 2010). Opie (2000) noted that safety problems occurred with β-blockers in a study with 12 550 hypertensive patients, those taking β-blockers had a 28% higher risk in developing diabetes whereas this was not observed in those treated with CCBs. He pointed out that the therapeutic option could depend both on the heart and on the patient. When treating an active middle-aged man, preservation of the quality of life must involved exercise training and sexual function. Therefore, there are good arguments for prescribing a CCB. An algorithm was produced for therapy of patients with stable ischemic heart disease (SIHD). A list of appropriate recommendations is available in the document (Fihn et al., 2012). When looking for a better control of heart rate, it is a reasonable combining β-blocker and CCB for angina. The Anglo Scandinavian Cardiac Outcome Trial (ASCOT) (Dahlof et al., 2005) and European Lacidipine Study on Atherosclerosis (ELSA) (Zanchetti et al., 2002) trial compared a β-blocker-based regimen to a CCB-based treatment. In ELSA, for a similar reduction in BP a better protection toward atherosclerosis was observed with CCB than with β-blocker. ASCOT was a multicenter, prospective, RCT comparing amlodipine to atenolol in 19,257 patients aged 40–79 years with hypertension and at least three other CV risk factors. The amlodipine-based regimen prevented more major CV events and induced less diabetes than the atenolol-based regimen. Thus, clinical studies favored CCBs for the management of hypertensive patients with SIDH.

The action of verapamil and diltiazem in stable effort angina

Verapamil was initially considered as a β-blocker (Kaltenbach and Zimmerman, 1968; Nayler et al., 1968), but was later characterized as a calcium antagonist by Fleckenstein et al. (1969). Several controlled clinical trials have established the efficacy of verapamil over placebo (Opie, 1989). Most of the controlled clinical trials (CRTs) dealt with comparison of verapamil vs. propranolol, using as an objective criteria as change in ST segment during and after exercise (Hopf et al., 1983). The usual procedure was to estimate the duration of a given work on the appearance of conventional electrocardiographic positivity (1 mm ST depression). As reported by de Ponti and Vincenzi (1981), the duration of work, expressed in second, increased significantly after verapamil administration and this effect was dose-dependent. Furthermore, after intravenous injection, the effective dose of verapamil was much lower than the dose required per oral route, an indication that the bioavailability of verapamil is reduced per os. In pharmacokinetic studies, the bioavailability of verapamil in human was estimated of 22% (Eichelbaum and Somogyi, 1983). This estimate was confirmed in functional studies, when the reduction of ST depression for a given work load was measured in patients treated with various doses of verapamil (Hopf et al., 1983).

Like nifedipine, it was compared with β-blockers by measuring the reduction of pain attacks or of nitroglycerin consumption. It appeared that verapamil 360 mg/day was equiactive to propranolol 300 mg/day (Sandler et al., 1968; Livesley et al., 1973; Johnson et al., 1981; Leon et al., 1981; Frishman et al., 1982; Sadick et al., 1982; Tan et al., 1982; Findlay et al., 1987). Since there is a dose-dependency for both agents, it is obvious that the superiority of one agent against the other cannot be estimated by comparing a single dose of each drug. When other criteria were used, it appeared that if β-blockers delayed ECG alterations more than angina, verapamil, and nifedipine delayed angina more than ECG alterations, indicating the existence of a qualitative difference between β-blockers and CCBs (de Ponti and Vincenzi, 1981).

Diltiazem has also been studied in stable angina and compared to placebo and propranolol. Effects observed were similar to verapamil. Bradycardia was observed like with verapamil, likely related to blockade of Ca_v3 channels (Striessnig, 2001).

Usually, those comparisons used diltiazem 9 mg four times daily, propranolol 60 mg four times daily against placebo. According to Schroeder et al. (1985), diltiazem decreased the sub-maximal and maximal degree of exercise-induced ST segment depression by over 50% compared to placebo ($P < 0.01$ vs. placebo). Diltiazem resulted in a higher exercise left ventricular ejection fraction compared to placebo, propranolol or the combination of diltiazem and propranolol (all less than $P < 0.05$). The sinus bradycardia occurred in patients required dose reduction.

In addition to chronic stable effort angina, the clinical use of CCBs for treatment of the other clinical types of ischemic heart disease including silent angina, vasospastic angina, unstable angina, and acute myocardial infarction are reported in recent Guidelines.

Side effects of verapamil, nifedipine, and diltiazem observed during studies reported above.

Drug-induced cardiac ischemia results from a sympathetic reflex in response to a rapid lowering of blood pressure, which

may provoke cardiac ischemia due to too fast IV administration. This has prompted a revision of the mode of administration of nifedipine, which is nowadays given by oral route in slow releasing (GITS) preparation. Earliest observations were the basis for the short-term controversy on the safety of CCBs discussed above. The more frequent side effects of nifedipine, verapamil, and diltiazem were related to their action on smooth muscles; with verapamil, about 70% of the patients complained of constipation; with nifedipine, the major side-effect was ankle edema, a side-effect also reported with diltiazem. Due to their potent negative inotropic effect, overdosage of verapamil and diltiazem have been avoided in order not to aggravate symptoms of cardiac insufficiency.

Calcium Channel Blockers in Cardiac Arrhythmias

In slow response tissues such as sinoatrial and atrioventricular nodes, non-dihydropyridine CCBs (nd-CCBs) do block Ca current that generate slowly propagating action potentials, this action displays antiarrhythmic effects. Acute myocardial infarction may convert fast conducting tissue such as ventricular myocardium and Purkinje fibers into slow response tissue. In ischemic areas, ionic changes cause partial depolarization in resting cells supporting slow Ca currents and leading to conduction blocks. Such blocks play a crucial role in the development of reentrant pathways. These processes are involved in the incidence of premature beats and ventricular tachycardia. In 1971 verapamil that was known to inhibit arrhythmia induced by ouabain, was used to reduce the ventricular rate in atrial fibrillation (Schamroth, 1971). It has been later ranged in class IV antiarrhythmics (Singh, 2000). Its action is due to interaction with intracellular binding sites different from the dihydropyridine receptor. Verapamil and diltiazem *in vivo* are powerful antiarrhythmic agents. Dihydropyridines usually evoke reflex tachycardia resulting from increase in sympathetic tone, masking their slight negative chronotropic effect. Verapamil and diltiazem are recommended in supraventricular tachycardia (Page et al., 2016). Intravenous verapamil produces the conversion of reentrant supraventricular tachycardia, diltiazem being less effective (Schamroth and Antman, 1983) and (Camm et al., 2010). Intravenous injection of verapamil and diltiazem in atrial fibrillation results in reduction of the ventricular response (Nademanee and Singh, 1988) an effect confirmed in the Verapamil plus antiarrhythmic drugs trial (De Simone et al., 2003). However, in the presence of anomalous bundle, verapamil, and diltiazem are contraindicated and the ESC guidelines recommend a treatment by catheter ablation for the management of atrial fibrillation (Camm et al., 2010).

Calcium Channel Blockers in Hypertension and HT Complications

According to 2016 reports of WHO and of Medscape, globally, the overall prevalence of raised blood pressure in adults aged 25 and over was around 40% in 2008. The number of hypertensive adults worldwide was estimated to 1.1 billion in 2015 with a disparity among countries, the prevalence being lowest in wealthy countries. This could be explained by diet and drug treatment control. In view of disability or death due to complications, such as cardiac ischemia, kidney insufficiency, stroke and dementia, it is mandatory to evaluate the efficacy of medications.

On the basis of their potent vasodilator properties, CCBs have been proposed as antihypertensive drugs, however CCBs may hardly be classified among vasodilators for their therapeutic action. Indeed, *in vivo*, they mainly act on the arterial bed and don't modify venous tone. As a consequence they don't evoke orthostatic hypotension. They reduce vascular resistance (and afterload) inducing a diminution of blood pressure (BP). At nifedipine therapeutic dosage, BP reduction is observed in patients with hypertension and not in normotensive individuals as shown on **Figure 6**. Further studies confirmed this selective BP effect with other CCBs including verapamil, nitrendipine, diltiazem, tiapamil, and isradipine but not with propranolol or captopril (Bühler et al., 1985). On the blood pressure of hypertensive SHR and normotensive WKY Knorr and Garthoff (1984) have compared the activity of nitrendipine and hydralazine. They found that the vasodilator hydralazine was equipotent in both strains, but that nitrendipine evoked a lower reduction of blood pressure in WKY than in SHR. The free cardiovascular tissue concentration of CCBs is similar in SHR and WKY after chronic oral treatment of respectively nisoldipine (80 mg/kg/day) and amlodipine (10 mg/kg/day). However, there is a significant reduction of BP in SHR and no change is observed in WKY (Godfraind et al., 1991; Morel and Godfraind, 1994). Such studies confirmed that considering their CV activity profile, CCBs are differing from classical vasodilators. Morel and Godfraind have shown that CCBs have a higher affinity for specific binding sites in vessels of hypertensive rats than in vessels of normotensive ones. The augmented sensitivity of BP to the effect of CCBs in hypertensive rats when compared to normotensive controls is likely due to this higher affinity. This increased affinity has been related to different levels of resting membrane potential of arteries leading to a different proportion of inactivated calcium channels (Morel and Godfraind, 1994). Furthermore, the CCB-dependent prevention of endothelial dysfunction in SHR vessels facilitates their relaxation resulting from calcium channel blockade (Krenek et al., 2001). CCBs efficacy has also been reported in patients with low renin activity whose hypertension is insensitive to β-blockers. Animal experiments show that the interaction of CCBs with the renin–angiotensin system is complex (Kyselovic et al., 2001). Like ACEI, CCBs exhibit a natriuretic effect. This action occurs without significant alteration in renal plasma flow or in glomerular filtration rate (Zanchetti and Stella, 1988). As shown by Honda et al., the natriuretic effect is related to the structure of the DHP-CCB: efonidipine, which mainly dilates afferent artery is more efficient than nifedipine, which mainly dilates efferent artery (Honda et al., 2001; Hayashi et al., 2007). This difference may be accounted for by interaction with T-type Ca channels (Hayashi et al., 2007), but other actions have been suggested. The natriuretic action supports the use of CCBs in monotherapy of hypertension. It is hidden during prolonged treatment but is manifested by a fall in natriuresis observed after drug withdrawal.

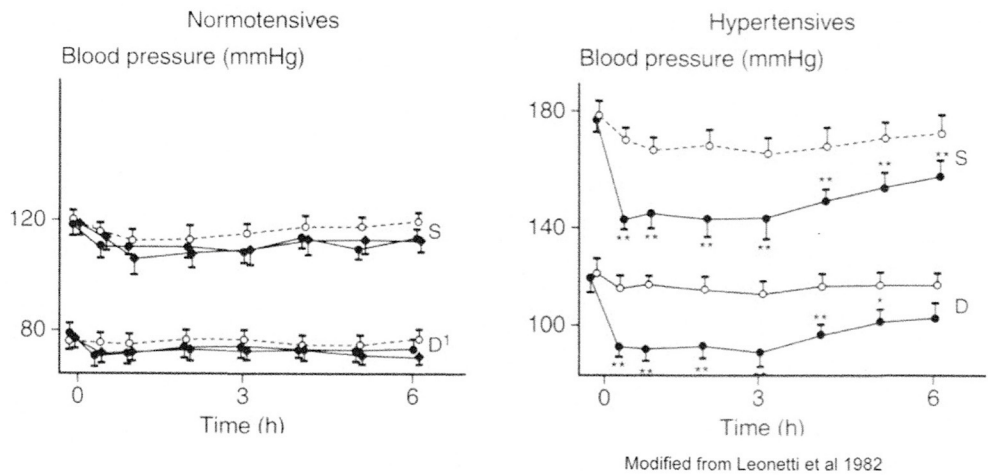

FIGURE 6 | Action of nifedipine on Blood Pressure: difference in normotensive and hypertensive patients. Modified from Leonetti et al. (1982).

CCBs are currently combined with other antihypertensive drugs such as ACEI or ARB for hypertension management (Godfraind, 2014). Long-term administration of CCBs to SHR opposes cardiac pathological remodeling and induces substantial regression of established LV hypertrophy thereby improving cardiac function (Kyselovic et al., 2001). Preserve trial was designed to compare enalapril and nifedipine on regression of LV hypertrophy at equivalent BP reduction (Devereux et al., 2001). Treatment began with enalapril 10 mg or nifedipine GITS 30 mg and matching placebo. If required, enalapril or nifedipine were increased to respectively 20 mg or 60 mg, hydrochlorothiazide (HCTZ; 25 mg) and then atenolol (25 mg) were supplemented if maximum dose did not control BP. More supplemental treatment with HCTZ was required in ACEI-treated patients than in CCB-treated ones. Importantly the study showed that both regimens similarly reduced to the normal range LV mass index and relative wall thickness during 1 year of treatment in about 50% of patients. The initial hypothesis was that normalization of BP and LV systolic load evoked regression or prevention of heart hypertrophy. However, several mechanisms might be involved (Godfraind, 2014). As demonstrated in various clinical trials (Tocci et al., 2015), BP lowering reduces the incidence of stroke and myocardial infarction. Head-to-head comparisons are incomplete but ASCOT trial (Sever et al., 2003) and several meta-analyses indicate that CCBs offer a better protection against stroke and myocardial infarction than angiotensin receptor blockers (Wang et al., 2007). This is consistent with survival to malignant hypertension of CCB-treated stroke-prone SHR (Godfraind and Salomone, 2015).

Alteration of renal function is another complication of hypertension. Diabetic nephropathy is particularly problematic. Verapamil and efonidipine (not marketed in United States) are equally efficient in this pathology (Sasaki et al., 2009). As pointed out above, studies on renal vessels showed that efonidipine dilates the efferent artery (Ozawa et al., 1999). This is attributed to a different action on T-type Ca channels. Verapamil act also at their level but amlodipine and nifedipine are weak blockers of those channels (Perez-Reyes et al., 2009). A role for amlodipine in renal disease is reported in combination therapies with other antihypertensive agents (Godfraind, 2014).

Hypertension impairs cognitive function, a harmful effect well-recognized nowadays (Moskowitz et al., 2010). It is clinically manifest as dementia that is recognized by cognitive decline eventually resulting in vascular dementia or Alzheimer disease globally diagnosed in about 40 million people. The evidence of the relation with hypertension has been hardly accepted because it requires longitudinal study (Staessen and Birkenhager, 2004). It is now believed that midlife hypertension has a deleterious influence on late-life cognitive function (Iadecola et al., 2016). Only few randomized clinical trials were conducted to study influence of antihypertensive therapy on the evolution of established dementia, their protocols were not similar regarding preceding clinical conditions and drugs used for treatment of selected patients. The first study reporting significant reduction of dementia incidence was a vascular dementia project part of the European Working Party on High Blood Pressure in the Elderly (SystEur) designed to investigate whether cardiovascular complications of isolated systolic hypertension in people aged 60 years or over could be reduced by nitrendipine (Staessen et al., 1997). The primary end point was fatal and non-fatal stroke. Because of the demonstration of a significant benefit for stroke, the trial initially planned for 4 years was limited to 2 years. During this period, the incidence of dementia was significantly reduced by 50%, expressed in cases per 1,000 patients-years, it was equal to 7.7 in the placebo group and to 3.7 in the nitrendipine group. The total number of cases was equal to 32. After this period, all subjects were invited to continue the trial for 2 year with the dosage of nitrendipine administered during the first period, which included patients previously treated with placebo. At the end of the period of 4 years, the total number

of dementia cases rose to 64. The group treated for 4 years with nitrendipine showed a significant lower number of dementia than the group initially treated by placebo, which had received nitrendipine during only 2 years, indicating a positive effect of the duration of nitrendipine therapy (Forette et al., 2002; Hanon and Forette, 2004). There is not yet confirmation of Syst-Eur study by longitudinal trial. A multicenter trial is in progress with nilvadipine in mild to moderate Alzheimer disease (Lawlor et al., 2015; Meulenbroek et al., 2016). However, there are published cross-sectional studies based on databases consultation. The most extensive is a Taiwan study consisting on analysis of the National Health Insurance Research Database dated from 2000 to 2010. It comprised 82,107 hypertensive patients aged 60 years or more. The annual incidence of dementia was 3.9 cases in the CCBs group vs. 6.9 per 1,000 persons-years in the comparator group ($P < 0.01$). Interestingly those data are not far from those of the longitudinal Syst-Eur study (Wu and Wen, 2016). The two other studies from databases are also confirmatory of the preventive action of CCBs in dementia (Feldman et al., 2016; Hwang et al., 2016). In the sub-study of the PROGRESS (Preventing Strokes by Lowering Blood Pressure in Patients With Cerebral Ischemia) trial, after treatment with ACEI and/or diuretics, it was shown that the risks of dementia and of cognitive decline among recurrent stroke patients were reduced to 34% and 45%, respectively, over mean time of 3.9 years, but those of non-recurrent stroke patients were not significantly affected (Tzourio et al., 2003). In SCOPE (The Study on Cognition and Prognosis in the Elderly), examination of cognitive function state with angiotensin II receptor blockade (ARB) against placebo showed no significant changes (Lithell et al., 2003). The effect of diuretics (perindopril plus indapamide) was examined in the HYVET-COG (Hypertension in the Very Elderly Trial assessing Cognitive decline and dementia incidence) study, the prevalence rate of dementia associated was reduced from the non-users but not significantly different after a mean of 2.2 years since the start of treatment. According to authors, this could be due to insufficiency of population tested (Peters et al., 2008).

It is obvious that further data are required for a robust evidence of the unique protective effect of CCBs treatment in prevention of dementia.

Calcium Channel Blockers in Atherosclerosis

The increase of plasma lipid levels constitutes risk factor for arteries. Clinical evidence indicates that the progression of the disease can be inhibited by sustained lipid-lowering therapy. As reported by Henry in a review on atherosclerosis (Henry, 1990), lesion formation is depending on calcium-regulated cellular processes such as chemotaxis, adhesion, migration, proliferation, lipid uptake, and necrosis. By acting on cell calcium uptake with calcium chelating agents, lanthanum trichloride and CCBs, atherogenesis in fat-fed animals may be retarded in the absence of hypolipidaemic effects. Fleckenstein and Colleagues confirmed those studies in fat-fed animals (Fleckenstein, 1987; Fleckenstein-Grun et al., 1992). Clinical studies were designed with CCBs in order to examine whether a similar result could be achieved in patients without influencing plasma lipid levels. The first was the International Nifedipine Trial on Antiatherosclerotic Therapy (INTACT) (Lichtlen et al., 1990). This trial showed significantly reduction of the appearance of newly formed coronary lesions in patients exposed to nifedipine. However, existing lesions were not modified. Unfortunately this trial didn't examine blood pressure. The latter information is needed to establish an action of CCB over other antihypertensive agents. Such requirement was included in the Verapamil in Hypertension and Atherosclerosis Study (Rosei et al., 1997; Zanchetti et al., 1998), which compared verapamil (240 mg OD) and chlortalidone (25 mg OD). Dissimilarities in carotid wall changes were small but greater incidence of CV events was noted in patients randomized to chlortalidone than to verapamil ($P < 0.05$). Differences in the incidence were paralleled by small differences in carotid wall changes suggesting that clinical and prognostic significance might depend on small effects on carotid plaques. In INSIGHT study, nifedipine GITS was significantly more efficient on intimal-media thickness than co-amilozide (hydrochlorothiazide + amiloride) (Rosenthal, 2002). Amlodipine effect on the progression of early coronary atherosclerosis was tested in PREVENT (Pitt et al., 2000) by evaluation of the rate of atherosclerosis in the carotid arteries together with monitoring the rates of clinical events. As compared to the placebo group, amlodipine had a significant effect in slowing the 36-month progression of carotid artery atherosclerosis but not in the rates of all-cause mortality or major cardiovascular events. Nevertheless, amlodipine use was associated with fewer cases of unstable angina. Other randomized trials including diuretics have confirmed that CCBs reduced progression of carotid lesions (Borhani et al., 1996; Simon et al., 2001). The ELSA trial (Zanchetti et al., 2002) compared carotid intimal-media thickness (IMT) changes over 4 years in patients receiving either a β-blocker or a dihydropyridine. Lacidipine exerted greater protection on carotid IMT progression and on number of new plaques increase than atenolol, despite a smaller BP reduction. In the analysis of the REGRESS Study Group trial, it appeared that co-administration of CCB, amlodipine or nifedipine with pravasatin caused the largest reduction in the appearance of new angiographic lesions, indicating that drug-combinations act synergistically and confirming the therapeutic action of CCBs in atherosclerosis (Jukema et al., 1996). This therapeutic action has been demonstrated in ApoE-deficient mice exposed to a lipid-rich Western-type diet (WD) (Nakashima et al., 1994), which even on a normal diet (ND) exhibit endothelial dysfunction (Plump et al., 1992; Bonthu et al., 1997; Barton et al., 1998; d'Uscio et al., 2001) proposed to initiate atherogenesis (Libby and Galis, 1995; Libby et al., 1995; Barton and Haudenschild, 2001). The latter is associated with increased peroxidation of plasma lipids, LDL and VLDL, as well as augmented susceptibility of lipoproteins to be oxidized *ex vivo* (Hayek et al., 1994).

In experiments from our group on ApoE KO mice (Kyselovic et al., 2005), lacidipine not only prevented endothelial dysfunction but also the development of atherosclerosis as shown in **Figure 7**, an action occurring without high cholesterol reduction. Indeed lacidipine treatment suppressed the loss of

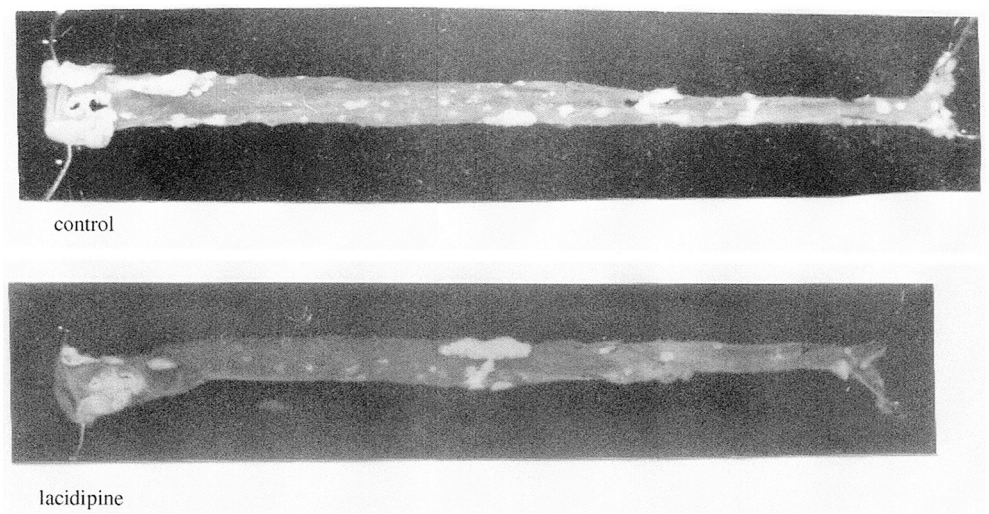

FIGURE 7 | Atherosclerotic lesions in aorta of apo-E deficient mice treated or not with lacidipine. Modified from Kyselovic et al. (2005).

acetylcholine-evoked relaxation in aorta of mice fed Western lipid-rich diet. It abolished increase in kidney TBARS markers of oxidative stress. The CCB treatment also normalized ET-1 plasma level and elevated PRA. A marked nephroprotective effect was related to decrease of oxidative stress and improvement of renal blood flow. Some effects reported in ApoE-deficient mice had also been observed in rabbit fed atherogenic diet (Becker et al., 1991).

Beyond the Cardiovascular System

Blockade of plasma membrane Ca entry through VOCs allowed therapeutic actions in neurological disturbances such as epilepsy, migraine, pain particularly neuropathic pain, and sub-arachnoids hemorrhage. When considering neuropharmacological indications, a review of 1986 (Godfraind et al., 1986a), mentioned flunarizine and nimodipine for treating common and classical migraine. Target of this action (cerebral arteries or neurones) remained contentious. Clinical trials examined the protecting action of CCBs from ischemic brain damage. In patients with aneurysmal subarachnoid hemorrhage, nimodipine secured patients at risk. Aged patients benefitted from cinnarizine and flunarizine for the treatment of vertigo and of sleep disorders. Some authors suggested a possible action in a number of forms of epilepsia. An interaction of neuroleptics with calcium channels has been clearly demonstrated (Santi et al., 2002). Therefore, calcium channels might be targets for therapeutic actions beyond the cardiovascular system.

There are experimental trends to extending the action of CCBs in cancer therapy by considering the major function of T-type currents in activating cancer cells (Heady et al., 2001; Panner and Wurster, 2006).

Migraine (with a Note on Vertigo)

As far as migraine treatment is concerned, there is a large body of information confirming the efficacy of flunarizine and nimodipine for the prevention of the migraine attacks, which are reduced in frequency in patients treated with these drugs (Formisano et al., 1991; Luo et al., 2012). Only a few studies include an additional follow-up after discontinuation of migraine prophylaxis with either drug. Nutti et al. reported a single blind evaluation of the efficacy and tolerance of flunarizine (25 patients) in comparison with nimodipine (25 patients) after discontinuation of a 6-month treatment (Nuti et al., 1996). It has also been reported that flunarizine reduced cortical spreading depression (Dora et al., 2003; Ayata et al., 2006), indicating activity on disseminated nervous disorders. Visual aura is the result of cortical spreading depression (CSD) that extends slowly across the cerebral cortex as a wave of neuronal depolarization (Bowyer et al., 2001; Hadjikhani et al., 2001). Headache is a consequence of neurogenic inflammation and activation of trigeminal nucleus caudalis and brainstem nuclei involved in the perception of pain (Moskowitz and Macfarlane, 1993; Waeber and Moskowitz, 2005). In the rat cortex CSD stimulates trigeminovascular afferents and evokes events consistent with the development of headache (Bolay et al., 2002). Thus, CSD is a critical event in the pathogenesis of migraine with aura. Some authors have proposed that nitrergic nerves are involved in this process (Olesen and Jansen-Olesen, 2000; Toda and Okamura, 2003). Mutations in the gene encoding the pore-forming α_1-subunit of $Ca_V2.1$ (voltage-gated P-Q-type) channels have been reported in familial hemiplegic migraine (FHM) (Tottene et al., 2002). They expressed FHM mutants in $Ca_V2.1$-deficient neurons. Therefore, a role for $Ca_V2.1$ channels could be dominant in the pathogenesis of migraine aura, indicating a potential molecular target for unspecific CCBs such as flunarizine. Alternatively, Ca_V1 channels (L-type) may also be involved, since they are upregulated in mouse brain subjected to episodes of CSD (Choudhuri et al., 2002). There are other targets, which could account for an action of CCBs (Reuter et al., 2002).

It appears that the precise mechanism of action of CCBs in migraine remains conjectural, but identified targets support the clinical observations of their therapeutic benefit (Pietrobon and Moskowitz, 2013).

Vertigo associated with migraine may also be reduced by CCBs (Hain and Uddin, 2003). Cinnarizine and nimodipine are efficacious in otological vertigo (Pianese et al., 2002). This is also true for the action of flunarizine in vestibular neuritis (Corvera et al., 2002). It might be that such therapeutics effects are related to a vascular action (Moskowitz and Macfarlane, 1993).

Neuropathic Pain

Pain is typically a sign of tissue injury and is usually temporary in duration. With healing, the pain associated with the wound will resolve. Its major role is to warn the individual from further injury. However, in some individuals, this painful experience can result in chronic pain that persists for months or even years after the initial insult. Abnormality of the peripheral, central, and sympathetic nervous system can result in a painful state termed neuropathic. Hence, neuropathic pain represents a chronic pain syndrome with a diverse etiology and perhaps an anatomical cause. L-, N-, and P/Q-types Ca^{2+} channel types are operating in the spinal cord and studies based on the spinal delivery of specific antagonists to high-threshold calcium channels reveal that their blockade, particularly of N-type channels, can prevent or attenuate subjective pain as well as primary and/or secondary hyperalgesia and allodynia. Selective block of N-type channels via intrathecal administration of ϖ-conotoxin GVIA or ϖ-conotoxin MVIIA significantly depresses pain, hyperalgesia, and allodynia in various animal models subjected to experimental situations (Vanegas and Schaible, 2000).

Ziconotide, a synthetic form of the *Conus magus* peptide toxin is a selective antagonist of the N-type Ca^{2+} channel. In animal models, it is antinociceptive of persistent, post-operative and neuropathic pain. It is more potent and specific than morphine on intrathecal administration and doesn't exhibit tolerance, acting on Ca^{2+}channels found in high concentration on the superficial laminae of the spinal cord dorsal horn. Pretreatment prevents allodynia and hyperalgesia (Penn and Paice, 2000; Ridgeway et al., 2000; Wang et al., 2000; Smith et al., 2002; Pope and Deer, 2015; Manda et al., 2016).

Gabapentin, 1-(aminomethyl) cyclohexaneacetic acid, is efficacious in epilepsy therapy through interaction with the alpha 2-delta subunit of L-type calcium channel (Striano and Striano, 2008).The gabapentin binding site has been purified from pig brain and gabapentin was identified as the first ligand to interact with the $\alpha_2\delta$ subunit of high-threshold Ca^{2+} channels (Dolphin, 2013). It is preventing hyperalgesia in a number of different models of neuropathic pain through its action at the post-synaptic dorsal horn and is effective in the treatment of neuropathic pain in diabetic neuropathy and post-herpetic neuralgia (Rose and Kam, 2002; Moore et al., 2014). Following sciatic nerve chronic injury, $[^3H]^+$gabapentin binding sites are upregulated in the dorsal horn. The association of $\alpha_2\delta$ with the pore-forming α_1 subunit of the Ca^{2+} channel modulates channel activity. This indicates that gabapentin could affect Ca channel function indirectly, thereby modifying neuronal excitability (Snutch et al., 2001). The exact mode of action of gabapentin still needs to be better evaluated. No doubt that this investigation shall improve our knowledge about interaction of drugs alleviating pain with calcium channels.

Subarachnoid Hemorrhage

Brain ischemia, which may be related to vasospasm, is a frequent cause of poor outcome in patients with subarachnoid hemorrhage (Kasuya et al., 2002; Lefranc et al., 2002), Experimental studies have indicated that nimodipine can prevent or reverse vasospasm in the nervous system (Hall and Wolf, 1986). In ischemic tissues, rundown of ionic gradients results in membrane depolarization allowing excessive Ca^{2+} influx that provokes a wide array of metabolic changes resulting in cell death. With reperfusion, extracellular Ca^{2+} increases, and rapid Ca^{2+} overload damages further cells (Siesjo, 1988a,b; Katsura et al., 2000). The most effective way to improve cell survival is blockade of the deleterious Ca^{2+} accumulation by CCBs, which promote the relaxation of cerebral vascular smooth muscle and thereby restore the cerebral blood flow (Ott et al., 2014; Hockel et al., 2016). The neuroprotective effect of CCBs has been documented after both global and focal ischemia in animal experiments (Bentahila et al., 1991; Alps, 1992; Oka et al., 1999, 2000). Studies with cells in culture have shown the ability of nervous cells to adapt to and recover from insult (Katsura et al., 1993). Neuronal and vascular smooth muscle cells are differently regulated by a chronic depolarization but are protected from the deleterious effects by nifedipine and nimodipine (Feron and Godfraind, 1995). Nimodipine is recommended in AHA guidelines for treating vasospasm following Subarachnoid Hemorrhage (Connolly et al., 2012).

CONCLUSIONS

In the various sections of this paper dedicated to history, facts reported by a large number of authors have been put forward. They do enlighten the global cooperative activity of the basic and clinical biomedical community to analysis of the potential of CCBs for treating a large spectrum of diseases from angina pectoris to various forms of dementia. There are some experimental discoveries that haven't been accounted for in this Review. They have been covered elsewhere under the topic "long-term effects" (Godfraind, 2000, 2004), which comprises antioxidant effects (Godfraind, 2005; Godfraind and Salomone, 2015), vascular remodeling actions (Arribas et al., 1999), gene expression and function of major autacoids including angiotensin (Kyselovic et al., 2001), NO (Krenek et al., 2001), endothelin (Godfraind, 2000). It is hypothesized that such long-term effects are involved in the therapeutic action of CCBs, an action that could not only been due to reduction of vascular tone controlling the level of blood pressure but also to BP independent actions. Authors of large RCTs reported above favor this assumption. On the basis of electrophysiological experimentation (Perez-Reyes et al., 2009), the hypothesis cannot be ruled out that in addition to blockade of $Ca_v1.2$, blockade of other voltage-operated channels could be of importance for therapy. However, as well as for long-term effects, robust

demonstration needs to be supported by clinical data. Much hope is provided by ongoing translational research (Griendling et al., 2016) and by Big Data analysis in the future of Medicines development.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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REVIEW
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A History of Drug Discovery for Treatment of Nausea and Vomiting and the Implications for Future Research

Gareth J. Sanger[1*†] *and Paul L. R. Andrews*[2†]

[1] *Blizard Institute and the National Centre for Bowel Research, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom,* [2] *Division of Biomedical Sciences, St George's University of London, London, United Kingdom*

The origins of the major classes of current anti-emetics are examined. Serendipity is a recurrent theme in discovery of their anti-emetic properties and repurposing from one indication to another is a continuing trend. Notably, the discoveries have occurred against a background of company mergers and changing anti-emetic requirements. Major drug classes include: (i) *Muscarinic receptor antagonists*–originated from historical accounts of plant extracts containing atropine and hyoscine with development stimulated by the need to prevent sea-sickness among soldiers during beach landings; (ii) *Histamine receptor antagonists*–searching for replacements for the anti-malaria drug quinine, in short supply because of wartime shipping blockade, facilitated the discovery of histamine (H_1) antagonists (e.g., dimenhydrinate), followed by serendipitous discovery of anti-emetic activity against motion sickness in a patient undergoing treatment for urticaria; (iii) *Phenothiazines and dopamine receptor antagonists*–investigations of their pharmacology as "sedatives" (e.g., chlorpromazine) implicated dopamine receptors in emesis, leading to development of selective dopamine (D_2) receptor antagonists (e.g., domperidone with poor ability to penetrate the blood-brain barrier) as anti-emetics in chemotherapy and surgery; (iv) *Metoclopramide and selective 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonists*–metoclopramide was initially assumed to act only via D_2 receptor antagonism but subsequently its gastric motility stimulant effect (proposed to contribute to the anti-emetic action) was shown to be due to 5-hydroxytryptamine$_4$ receptor agonism. Pre-clinical studies showed that anti-emetic efficacy against the newly-introduced, highly emetic, chemotherapeutic agent cisplatin was due to antagonism at 5-HT_3 receptors. The latter led to identification of selective 5-HT_3 receptor antagonists (e.g., granisetron), a major breakthrough in treatment of chemotherapy-induced emesis; (v) *Neurokinin$_1$ receptor antagonists*–antagonists of the actions of substance P were developed as analgesics but pre-clinical studies identified broad-spectrum anti-emetic effects; clinical studies showed particular efficacy in the delayed phase of chemotherapy-induced emesis. Finally, the repurposing of different drugs for treatment of nausea and vomiting is examined, particularly during palliative

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****Correspondence:***
Gareth J. Sanger
g.sanger@qmul.ac.uk

† These authors have contributed equally to this work

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care, and also the challenges in identifying novel anti-emetic drugs, particularly for treatment of nausea as compared to vomiting. We consider the lessons from the past for the future and ask why there has not been a major breakthrough in the last 20 years.

Keywords: nausea and vomiting, drug discovery, metoclopramide, histamine H_1 receptor antagonists, muscarinic receptor antagonists, 5-hydroxytryptamine$_3$ receptor antagonists, neurokinin$_1$ receptor antagonists, olanzapine

INTRODUCTION

The sensation of nausea and the ability to vomit are key components of human defenses against unintentional ingestion of noxious material and are part of a hierarchically organized defensive system (**Figure 1**; Davis et al., 1986; Stern et al., 2011). Ingested toxins must be detected rapidly and reliably, nausea induced quickly to limit further ingestion, and vomiting initiated promptly to void contaminated ingested material whilst still in the lumen of the upper digestive tract.

Nausea is considered a "warning." It can be considered to represent "low intensity" stimulation of afferent pathways, which if activated more intensely, trigger vomiting, yet paradoxically, it is considered easier to prevent vomiting rather than nausea by anti-emetic drugs (Andrews and Sanger, 2014). Likewise, risk factors for induction of nausea as opposed to vomiting may also differ, as exemplified by post-operative nausea and vomiting (Stadler et al., 2003). An accepted function of nausea is that it causes a learned aversion to the food associated with the nausea, leading to avoidance when subsequently encountered, sometimes lifelong (Stern et al., 2011).

The pathways which evolved to detect ingested toxins and aberrant motion can also be triggered by diverse diseases and pharmacological therapies (**Figure 2**). Thus, nausea and vomiting rather than being adaptive responses of evolutionary significance (arguably including pregnancy sickness in humans; Profet, 1988, 1992; Flaxman and Sherman, 2000 but for a different view see Brown et al., 1997; Weigel et al., 2006) become "symptoms of disease" or "side-effects of drugs" which often require treatment (**Figure 2**). Motion sickness, pregnancy sickness and adverse effects of therapy (primarily for cancer) have driven the development of anti-emetic drugs since the early 1940s.

Anti-emetics are sometimes viewed as a niche therapeutic area but this is incorrect as: (a) Nausea and vomiting are amongst the most common reasons for an emergency department visit

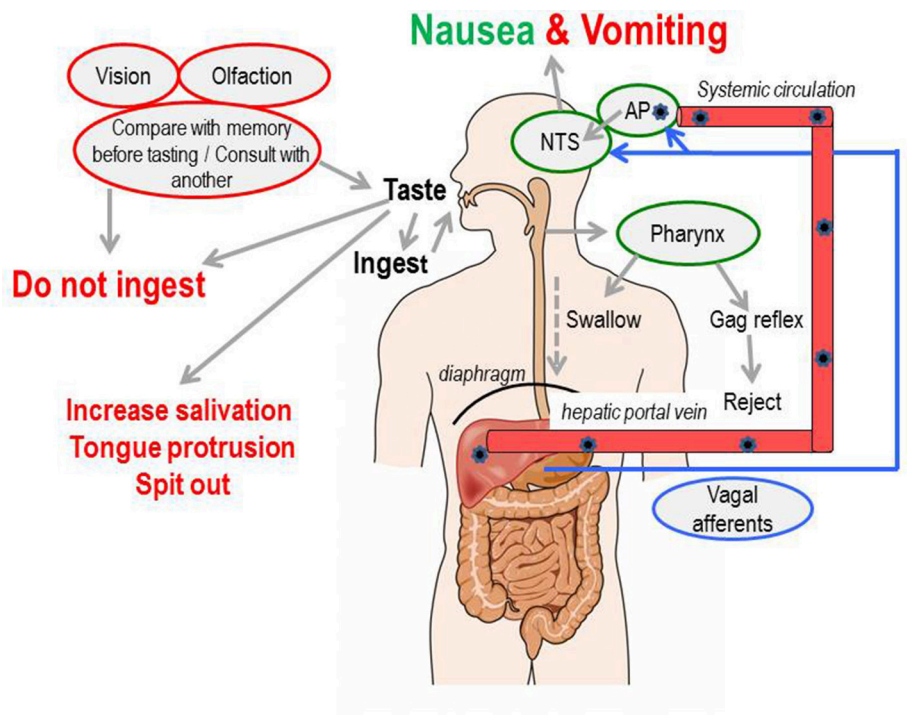

FIGURE 1 | A summary of the levels of defense employed to initially avoid and, if required, to detect and respond to toxins ingested with the food. AP, area postrema (also known as the "chemoreceptor trigger zone" for emesis, but see text for discussion); NTS, nucleus tractus solitarius; the site in the dorsal brainstem where inputs from the vagal afferents and the area postrema are integrated and from which outputs pass to other areas of the brainstem to coordinate the motor outputs for vomiting and from which information is relayed to "higher" brain regions to evoke the sensation of nausea. Figure adapted and modified from Andrews (1993).

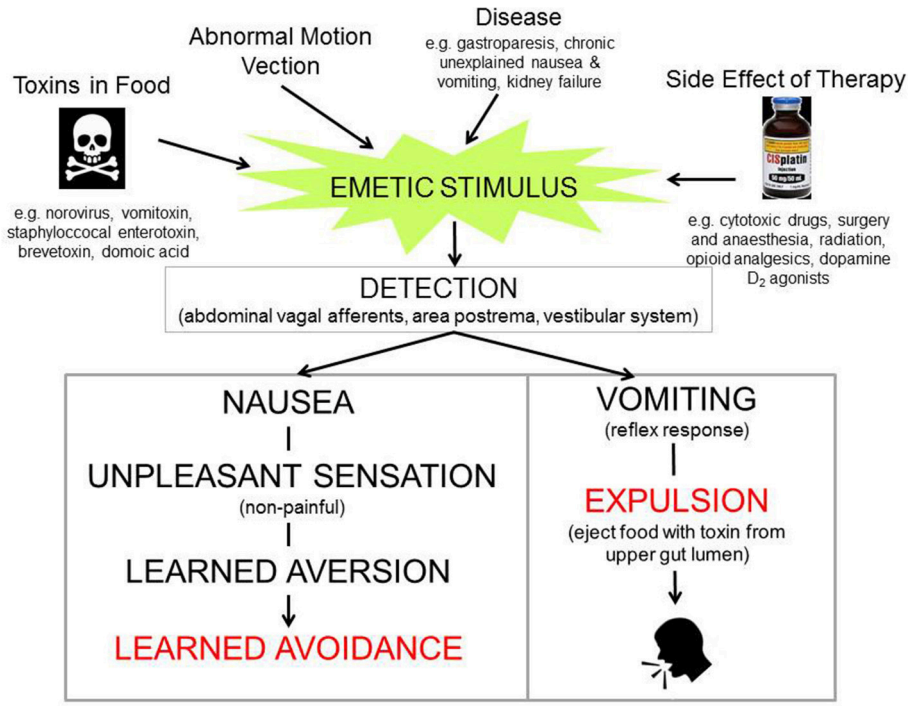

FIGURE 2 | Diagram illustrating that nausea and vomiting can be evoked by stimuli ranging from toxins in the food where they may be viewed as an "appropriate" response helping to defend the animal, to diseases and therapeutic interventions where they are viewed as undesirable and are classified as "symptoms" or "side-effects." Profile of the head from http://getdrawings.com/talking-head-silhouette.

(Meek et al., 2015), (b) An anti-emetic (ondansetron) was on the list of drugs with sales of one billion $US a year before patent expiry and together with metoclopramide (an anti-emetic and gastric prokinetic drug), ondansetron has been included on the World Health Organization list of essential medicines[1], (c) Developments in anti-emetics (particularly antagonists at 5-hydroxytryptamine$_3$ receptors; 5-HT$_3$) were included in the "top five advances" in modern oncology in a 2014 American Society for Clinical Oncology survey[2], (d) Anti-emetics decrease overall healthcare costs in cancer patients because they enable treatment in day centers and reduce the need for hospitalization following severe vomiting; a similar argument applies to post-operative nausea and vomiting (PONV), reducing the need for longer (particularly overnight) hospitalization, (e) Anti-emetics provide rare examples of clinical agents acting as an antagonist at a ligand-gated ion channel (5-HT$_3$) and at a receptor for a peptide (neurokinin$_1$; NK$_1$), (f) Significant conditions remain in which nausea represents a defining but poorly-treated symptom in large patient populations (e.g., palliative care, gastroparesis, functional dyspepsia).

In this review, current nomenclature is used[3] to describe G-Protein Coupled Receptors (GPCRs) and ion channels. Nevertheless, it is important to appreciate that when many anti-emetic drugs were discovered, their target GPCR or ion channel was not fully characterized or even defined. Progress in understanding anti-emetic drug physiology and receptor pharmacology can therefore be viewed as running in parallel with characterization of these targets. Such progress also illustrates the evolution in methods of drug discovery, from early reliance on animals to define therapeutic and adverse effects of drug candidates, through to the use of such models to define novel receptor functions (e.g., 5-HT$_3$ receptor) and today's focus on recombinant human receptors to characterize compound activity before translation using animals and humans. The last 30 years in particular, have also seen major re-organizations of the pharmaceutical industry. **Figure 3** shows the companies which played significant roles in anti-emetic drug discovery, many of which disappeared during mergers and takeovers, impacting research. **Table 1** provides details of key contributions.

This review outlines the mechanisms of nausea and vomiting, providing a background to the discovery and pharmacology of licensed anti-emetic drugs and compounds still in clinical development. We examine the shifting strategies adopted by the pharmaceutical industry and academia over the last ∼75

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[2]https://www.asco.org/about-asco/press-center/news-releases/asco-50th-anniversary-poll-names-top-5-advances-past-50-years

[3]http://www.guidetopharmacology.org/

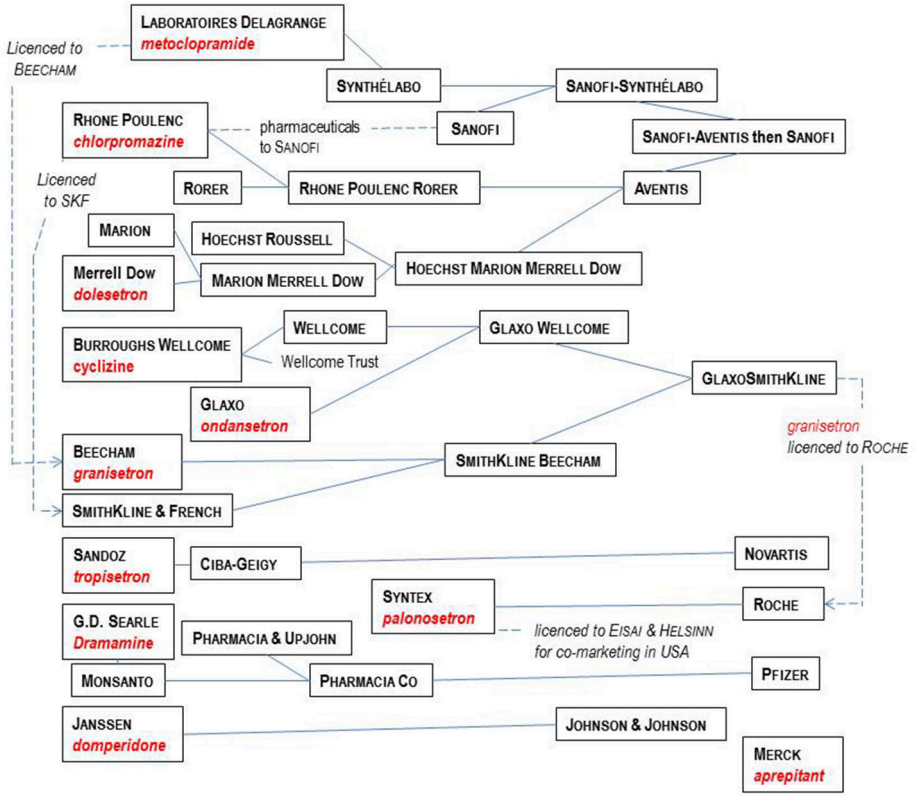


FIGURE 3 | Major players in the pharmaceutical industry responsible for the development of the main anti-emetic drugs over the time course covered by this review. See text for details and references.

years. Lessons learned and challenges to further advances are also highlighted, together with current research trends.

COMMON CAUSES OF NAUSEA AND VOMITING

The three main causes of nausea and vomiting which may require therapeutic intervention are diseases (organic and functional), drug or other therapies (e.g., PONV) and motion sickness. Amongst the diseases, digestive tract disorders are currently being investigated most actively, with interest focused on the genesis of nausea in conditions such as gastroparesis (see below). Treatment of the emetic side effects of anti-cancer chemotherapy (Andrews and Rudd, 2016), analgesics in palliative care (Smith and Laufer, 2014) and PONV (Horn et al., 2014) are the commonest examples in the "side-effect of therapy" category (for reviews see Stern et al., 2011; Koch and Hasler, 2017) but it should also be noted that nausea and vomiting are surprisingly common side-effects of drugs in general; the Electronic Medicines Compendium indicates nausea as an adverse event for >50% of a wide range of drugs and both nausea and vomiting for >33% (Lee, 2006). Indeed, as an adverse event, nausea and vomiting is second only to the potential for abuse liability in their impact on development of new chemical entities (NCEs) as therapeutic agents (Holmes et al., 2009), so predicting such liability early in the discovery process is of high importance. Meta-analysis and database mining of "historic" animal and human studies (which may never be repeated) provide a useful approach to identification of chemical templates most likely to induce vomiting (Parkinson et al., 2012; Percie du Sert et al., 2012).

Motion sickness is not a disease but, apart from food poisoning and pregnancy, it is probably the most likely cause of nausea and vomiting experienced by readers of this review. Medications used for travel sickness (e.g., Joy Rides® and Kwells® [formulations of hyoscine hydrobromide]; Stugeron® [cinnarizine]) are rare examples (in the UK) of widely-used anti-emetics available without prescription. Interest in motion sickness continues because of "space motion sickness," occurring in ∼70% of astronauts during the first 3 days in space (Crampton, 1990; Weerts et al., 2015).

CLINICAL NEED FOR ANTI-EMETIC DRUGS

Vomiting has a diverse range of potential impacts upon the person involved and also potentially on others. The consequences are psychological (e.g., demeaning), physical (e.g., chronic fatigue

TABLE 1 | The major pharmaceutical companies involved in the discovery of anti-emetic drugs during the period covered by this review and a summary of their key contribution to the area.

Rhône-Poulenc Laboratories
• Rapidly focussed on the therapeutic potential of the newly-discovered "antihistamines," searching libraries of compounds originally synthesized for another use. The first antihistamine to treat anaphylaxis and allergic reactions was phenbenzamine, introduced into the clinic in 1942.
• Re-examination of the antihistamines to optimize the "anti-shock" property led to synthesis in 1946 of chlorpromazine (4560-R.P). This compound had low antihistamine activity but in 1951 the company demonstrated its ability to prevent emesis evoked by apomorphine in dogs.
G.D. Searle & Co
• Introduced the "antihistamine" dimenhydrinate (Dramamine), a combination of diphenhydramine and 8-chlorotheophylline (a mild stimulant and derivative of theophylline) as a counter measure against the drowsiness, somnolence, and sedation caused by H_1 receptor antagonism within the brain.
Burroughs Wellcome
• Developed the "antihistamine" cyclizine, in 1947, subsequently taken on the Apollo moon missions as a treatment for space sickness.
Laboratoires Delagrange
• Identified metoclopramide in the mid-1950s, during a programme aimed at improving the properties of procainamide, a cardiac anti-arrhythmic and local anesthetic drug derived from procaine. The drug had negligible local anesthetic or cardiac anti-arrhythmic activity but an ability to inhibit emesis in dogs evoked by multiple stimuli. Soon after, metoclopramide was also found to stimulate GI motility and reduce symptoms associated with various upper GI disorders.
Janssen Pharmaceutica
• Among the antipsychotic compounds the company had developed in the mid-1950s, some were effective antagonists at the dopamine receptors in the chemoreceptor trigger zone, an area of brain outside the blood-brain barrier, involved in regulation of vomiting. Domperidone was identified in 1974 as an antagonist which did not cross the blood-brain barrier and hence, less likely to evoke the extrapyramidal side-effects.
Merrell Dow
• Synthesized MDL72222 from the chemical template of cocaine, the first selective 5-HT_3 receptor antagonist, originally aimed at the treatment of migraine. A later compound (MDL73147 or dolasetron) was marketed for the control for chemo-radiotherapy-induced emesis.
Beecham Pharmaceuticals
• Identified the anti-emetic activity of the 5-HT_3 receptor antagonists, developing its own molecule (BRL43694 or granisetron, launched by SmithKline Beecham for the control of chemoradiotherapy-induced emesis) and successfully filed a patent to cover the anti-emetic use of Glaxo's compound (GR38032F or ondansetron), originally designed for treatment of "a variety of disorders including migraine" before being specifically patented for treatment of depression, schizophrenia, anxiety, and cognitive disorders.
Glaxo
• Identified ondansetron for the treatment of migraine and a variety of CNS disorders. Subsequent marketing as an anti-emetic drug incurred royalty payments to Beecham/SmithKline Beecham who owned the patent covering the anti-emetic use of this drug.
Sandoz
• Identified the 5-HT_3 receptor antagonist ICS 205-930 (tropisetron), originally for treatment of migraine, subsequently sponsoring research to characterize its anti-emetic activity and "re-purpose" for treatment of chemoradiotherapy-induced emesis.
Merck
• Aprepitant introduced in 2003, following initial characterization for treatment of depression and emesis and a long history of failure of other NK_1 receptor antagonists to treat pain.
Syntex Discovery Research
• Synthesized and characterized palonosetron (RS 25259-197), licensed to Eisai and Helsinn for co-marketing in the USA in 2003 (the same year as aprepitant).

See text for further details.

and fractured ribs), metabolic (e.g., dehydration, anorexia) and when caused by medications, can affect therapeutic outcomes (e.g., if treatments are refused); these are summarized in **Figure 4**. In circumstances when the vomiting is not induced by food-borne toxins, blockade by an anti-emetic drug is desirable. Notably, although vomiting is unpleasant, patients are frequently more concerned about nausea, because as with chronic pain, it can be unremitting. In contrast, vomiting occurs in episodes, albeit sometimes spread over many days. Further, the adaptive function of nausea (learning to avoid foods that caused its induction on a previous encounter) becomes a liability when it leads to avoidance and refusal of potentially curative therapy in the case of some anti-cancer chemotherapy (Maceira et al., 2012).

This review focuses on the identification of anti-emetic drugs for therapeutic use in humans. Not discussed are important veterinary applications, particularly in oncology (Kenward et al., 2017).

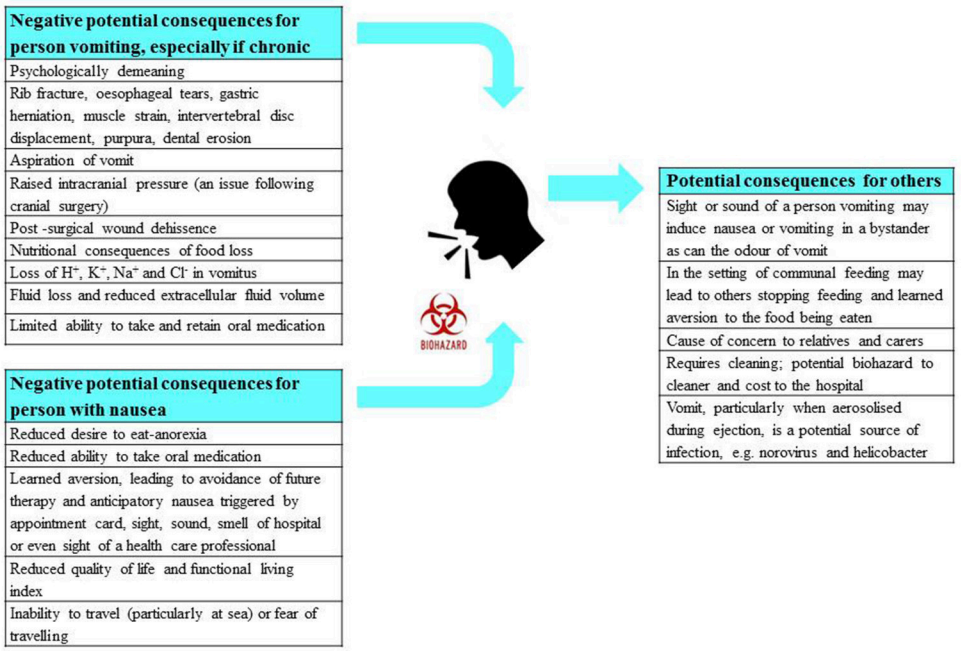


FIGURE 4 | A summary of the physical, physiological, and psychological consequences of nausea and vomiting for the person suffering, as well as for any observers including health care professionals. The potential risk of infection from vomiting is also highlighted. Profile of the head from http://getdrawings.com/talking-head-silhouette.

BRIEF INTRODUCTION TO MECHANISMS

The pathways involved both in the induction and the motor outputs of emesis are briefly described, so the sites of action of anti-emetics (discussed below) can be identified (**Figure 5**).

Major Pathways

Vestibular System

Although motion sickness can be induced by the vestibular system alone (Irwin 1881; the first person to use the term "motion sickness"), it more often involves conflicting or discordant signals from the vestibular and visual systems, possibly with involvement of proprioceptive inputs (Money, 1970; Reason, 1978; Oman, 2012; Lackner, 2014; Yates et al., 2014; Golding and Gresty, 2015; Bertolini and Strauman, 2016).

Area Postrema (AP)

Located at the caudal extremity of the IVth ventricle, the area postrema is characterized by relatively permeable blood-brain and cerebrospinal fluid-brain barriers. Lesion studies primarily in the 1950s and 1960s (e.g., Wang and Borison, 1952; for review see Borison, 1989) implicated this region in the emetic response to a diverse range of substances in the blood and led to its description as the "chemoreceptor trigger zone" (CTZ) for emesis. A widespread view then developed that agents in the circulation could *only* induce emesis via the AP, resulting in this region becoming a focal point for therapeutic intervention (see Domperidone, below) and distracting attention from the involvement of other pathways activated by systemic agents. Nevertheless, the importance of the AP is exemplified by its role in the induction of emesis by a number of endogenous circulating agents (e.g., adrenaline, glucagon-like peptide-1, cholecystokinin) as well as by drugs (e.g., apomorphine, digoxin, morphine; see Stern et al., 2011). The reliable activation of emesis by apomorphine via the AP led to its widespread use as a test stimulus for investigating potential anti-emetic agents but over-simplistic interpretation of the blockade of apomorphine-induced emesis by candidate drugs may have led to erroneous conclusions as illustrated by a quotation from Borison and McCarthy (1983, p. 16): "A misconception of the emetic mechanism that has led to false critical expectations is the idea that experimental drug antagonism of apomorphine-induced vomiting is equivalent to general inactivation of the chemoreceptor trigger zone."

Abdominal Vagal Afferents

Projecting from the stomach and small intestine, vagal afferent neurons send information to the brain stem about the mechanical activity of the muscle and the chemical nature of the luminal environment. This includes the effects of distension, particularly of the gastric antrum and duodenum, which can induce nausea and vomiting but paradoxically, gastric motor quiescence is also associated with nausea (Sanger et al., 2013). Increasing evidence also points toward dysrhythmic gastric movements in certain conditions associated with nausea (e.g., gastroparesis) thought to be detected by vagal mechanoreceptors and signaled to the brainstem (Stern et al., 2011). In addition, the mucosal chemoreceptive vagal afferents are implicated in emesis caused

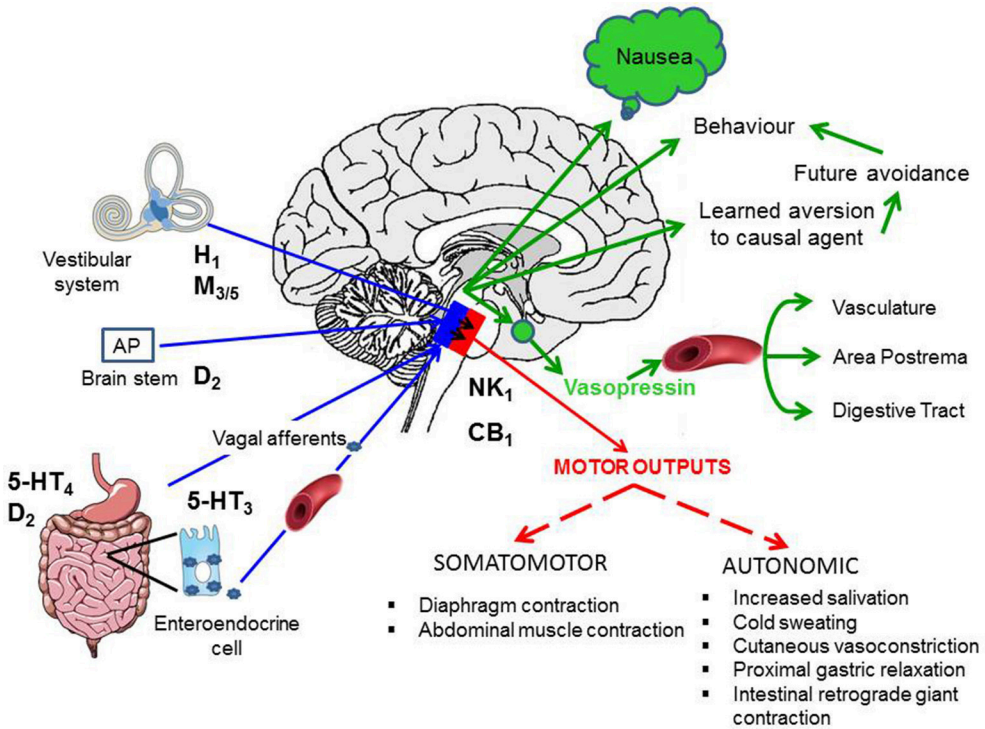


FIGURE 5 | Summary of the pathways responsible for the induction of nausea and vomiting (blue arrows), the integrative regions in the brain stem (blue box indicates dorsal brain stem and nucleus tractus solitarius in particular) and the output pathways for nausea (green) and the motor outputs for vomiting (red box indicates the pathways in the ventral brain stem). See text for details of pathways. CB_1, cannabinoid$_1$ receptor; D_2, dopamine$_2$ receptor; H_1, histamine$_1$ receptor; $M_{3/5}$, muscarinic$_{3/5}$ acetylcholine receptor; 5-HT_3-5-hydroxytryptamine$_3$ receptor; 5-HT_4-5-hydroxytryptamine$_4$ receptor; NK_1, tachykinin neurokinin $_1$ receptor. Adapted and modified from Stern et al. (2011).

by ingested luminal toxins and irritants. In this setting, the detection of substances in the lumen is via enteroendocrine cells within the mucosa, which release neuroactive substances (e.g., 5-HT, cholecystokinin) locally to activate receptors on the vagal afferents terminating in close proximity. Based upon direct and circumstantial evidence, Andrews et al. (1988) proposed that the enteroendocrine cells and the vagal afferents were involved in the acute emetic response to anti-cancer chemotherapeutic agents (e.g., cisplatin, cyclophosphamide) and abdominal radiation by the release of 5-HT (and other substances; see below) from the cells to act at 5-HT_3 receptors on the vagal afferent terminals (see Andrews and Rudd, 2016 for review).

Motor Outputs

Vomiting

Vomiting is a reflex motor event coordinated in the brainstem. Classically, the term "vomiting center" described the brainstem locus from which vomiting could be induced when stimulated and was viewed as a conceptual target for anti-emetic drugs (Wang and Borison, 1950). Although "vomiting center" is a useful concept and is still used in text books (e.g., Rang and Dale's Pharmacology; Ritter et al., 2016), as the network of brainstem nuclei [e.g., nucleus tractus solitarius (NTS), dorsal motor vagal nucleus, Bötzinger complex] responsible for the genesis and coordination of the retching and vomiting motor pattern have been identified (Hornby 2001), such "black box" descriptions of networks may become redundant.

Key events in vomiting are: (a) Relaxation of the proximal stomach via reciprocal changes in activity of vagal inhibitory and excitatory neurons, together with a retrograde giant contraction (RGC) beginning in the lower small intestine and progressing to the stomach under vagal control (Lang, 2016). These changes confine potentially-contaminated gastric content to the stomach (the only place from which ejection by vomiting is possible) and the RGC returns already-emptied contents to the stomach. Retching only begins once the RGC reaches the stomach; (b) Contraction of the hiatal region of the diaphragm and inhibition of the crural diaphragm surrounding the lower esophagus by the phrenic nerve, and contraction of the abdominal muscles by the spinal motor neurons. It is these motor events which in terrestrial mammals provide the propulsive force for oral ejection of gastric contents (see Stern et al., 2011; Koch and Hasler, 2017).

Nausea

Compared with vomiting, nausea is poorly understood and difficult to define operationally (Stern et al., 2011; Balaban and Yates, 2017). There are, for example, fewer than 10 published human brain imaging studies investigating brain activity during nausea and all but one (Miller et al., 1996) used illusory self-motion as the stimulus. These studies implicate the anterior

cingulate cortex ("visceromotor cortex"), inferior frontal gyrus, insular cortex and amygdala (Napadow et al., 2012; Farmer et al., 2015; Sclocco et al., 2016). In some brain areas (e.g., posterior cingulate cortex) the activity showed a negative correlation with nausea (Farmer et al., 2015). However, it must be emphasized that we do not yet know which regions are associated with the genesis of nausea and which are associated with the emotional and stressful aspects of the sensation and hence, are implicated in the associated autonomic changes characterized by increased sympathetic outflow. For a detailed review of the central pathways implicated in nausea, see Stern et al. (2011) and Koch and Hasler (2017).

Healthy volunteers and patients reporting nausea also have a number of physiological changes often referred to as "prodromata of vomiting." The main ones are cold sweating (forehead) and pale skin pallor due to regional cutaneous vasoconstriction, tachycardia and increased heart rate variability, elevated plasma vasopressin (but not oxytocin) concentration indicative of hypothalamic-posterior pituitary involvement, and inhibition of gastric motility (see Stern et al., 2011, and Koch and Hasler, 2017).

The relatively poor temporal resolution of studies which have attempted to correlate physiological changes with the subject's reporting of nausea means that for elevated plasma vasopressin, gastric dysrhythmia and delayed gastric emptying, there is debate about the extent to which each contributes to the genesis of the sensation of nausea or are simply a component of the physiological response to activation of the emetic pathways (Stern et al., 2011; Andrews and Sanger, 2014). Resolving this "cause-consequence" conundrum is important for identifying which patient groups require therapeutic approaches that are directed centrally or peripherally.

Nausea is recognized as poorly treated in comparison to vomiting (Andrews and Sanger, 2014) and has been described as a "neglected symptom" during treatment of cancer patients (e.g., Foubert and Vaessen, 2005; Greaves et al., 2009; Jones et al., 2011). It is also one of the defining symptoms in the common, poorly-treated conditions of gastroparesis, functional dyspepsia and chronic unexplained nausea and vomiting (Sanger and Pasricha, 2017). However, such prevalence does not seem to have stimulated research to improve our understanding of the pathways involved in the etiology of nausea.

EARLY SERENDIPIDOUS DISCOVERIES OF ANTI-EMETIC DRUGS

The original drive to identify anti-emetic drugs most likely originated with the desire to block sea-sickness, with references to treatments in Classical Greek and Roman literature (Huppert et al., 2016) and more recently, Shakespeare (Cymbeline III, iv, 186; Kail, 1986). These and later attempts to block nausea and vomiting prior to and during World War II (WWII, 1939–1945) were largely based on traditional, historic and unproven remedies for sea-sickness, with more than 40 treatments identified based on publications in the Lancet between 1828 and 1928 (Reason and Brand, 1975). The only substances recognized in antiquity and pre-WWII and shown subsequently to have efficacy, are atropine and hyoscine (see below). This required development of methodologies for objective assessment of sea-sickness at sea and methods for induction of motion sickness in controlled laboratory conditions in humans and animals (McEachern et al., 1942; Noble, 1945; Babkin et al., 1946; Holling, 1947; Brand and Perry, 1966). The drug trials methodology developed by the United Kingdom military and the Medical Research Council became a model for drug trials in other areas.

By 1976, a series of largely serendipitous developments identified four categories of anti-emetic drug (Gibbs 1976): (i) Anticholinergic drugs (later shown to antagonize muscarinic M_3 and M_5 receptors); (ii) Antihistamines (later shown to act predominantly as antagonists at the histamine H_1 receptor but also at muscarinic receptors); (iii) Derivatives of phenothiazine (shown to act as dopamine D_2 receptor antagonists but also with effects at other receptors); (iv) Metoclopramide, a drug derived from the local anesthetic procainamide (initially described as a D_2 receptor antagonist before other activities were discovered some year's later; see below).

The early discoveries were made by testing in humans to confirm anecdotal reports (e.g., the anti-cholinergic hyoscine) or after rapid transition of a newly discovered molecule into the clinic, when anti-emetic activity was unintentionally discovered (e.g., antihistamines). Thereafter, animal studies began to appear more frequently, beginning with their use in the discovery of anti-emetic activity during routine screening for general activity (the phenothiazines) and then to characterize the actions of other dopamine$_2$ (D_2) receptor antagonists from chemical programmes initially directed at controlling psychiatric disorders.

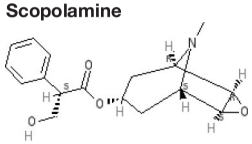
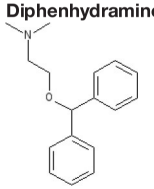
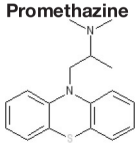
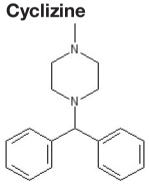
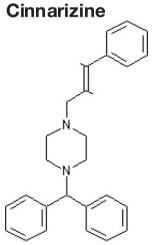
The pharmacology (receptor affinities/potencies) and structures of the major anti-emetic drugs discussed in the sections below are summarized in **Table 2**.

Hyoscine and Scopolamine

The alkaloids hyoscyamine and hyoscine (also known as scopolamine) are found in different plants from the family *Solanaceae* (e.g., hyoscyamine in the deadly nightshade *Atropa belladonna* and hyoscine from henbane, *Hysoscyamus niger* (Henry, 1939). The toxic and medicinal properties of this plant family have been known since antiquity (see Thearle and Pearn, 1982). Extraction of the naturally-occurring levorotatory isomer of hyoscyamine leads to formation of the racemic mixture known as atropine (Sneader, 2005).

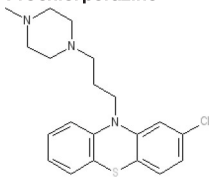
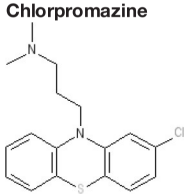
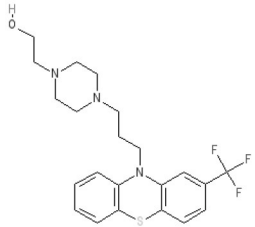
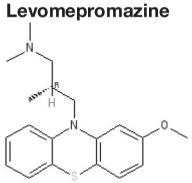
Although this class of drug was suggested to be effective against seasickness as long ago as 1881 (Irwin, 1881) it was not until WWII that structured trials investigated the activity of potential anti-emetic medications including hyoscine, atropine, the different enantiomers of hyoscyamine, phenobarbitone, sodium hydantoinate, chloretone, syntropan, hexobarbitone, and methidrine (Reason and Brand, 1975). The trials occurred using mine sweepers sent to sea in rough weather and positive responders were those who did not experience nausea and/or vomiting. The results, for the first time, demonstrated the preventative efficacy of hyoscine in particular and also atropine and the l-isomer of hyoscyamine (Holling et al., 1944; Holling,

TABLE 2 | Structures, receptor affinities, and actions of anti-emetic drugs.

	D_1	D_2	D_3	D_4	α_1	α_2	H_1	M	5-HT_{2A}	5-HT_3A	Other
Muscarinic Receptor Antagonist											
Scopolamine 		*K_i >10,000 nM (rat)*[13]					*K_i >10,000 nM (rat)*[13]	M_1 **9.0** M_2 **8.7** M_3 **9.4** M_4 **9.5** antagonist			
Histamine H_1 Receptor Antagonists											
Diphenhydramine 		*K_i >10,000 nM (rat)*[13]					**7.9** antagonist	*pA_2 7.1 antagonist (rat)*[7]			
Promethazine 		*K_i 240 nM (rat)*[13]					**9.6** antagonist	*K_i 21 nM (rat)*[13]			
Cyclizine 							**8.4** antagonist				*Pre-ganglionic cholinergic inhibition (animals)*[15]
Cinnarizine 							*antagonist*[16]	*antagonist*[16]			*Blocks L-type and T-type calcium channels*[16]

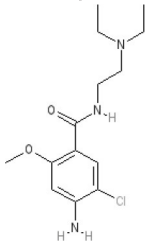
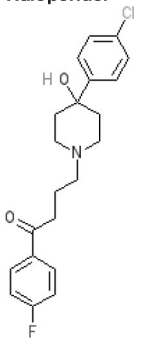
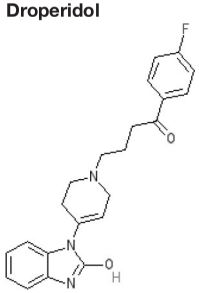
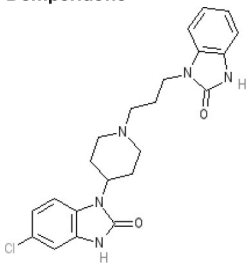
(Continued)

TABLE 2 | Continued

	D_1	D_2	D_3	D_4	α_1	α_2	H_1	M	5-HT_{2A}	5-HT_3A	Other
Phenothiazines											
Prochlorperazine 	7.1 antagonist	**8.4** antagonist	**8.4** antagonist	6.1 antagonist	*K_i 200 nM (rat)*[14]		*pIC_{50} 6.7 inverse agonist*[6] *8.2*[8]	*K_i 2,100 nM (rat)*[13]	***8.2***[3]	*Inactive (rat)*[10]	
Chlorpromazine 	7.1 antagonist	7.0–7.6 antagonist	7.2–7.5 antagonist	7.8 antagonist	*α_{1A} K_i 0.28 nM*[12]	α_{2A} 5.9–6.6 α_{2B} 7.2–8.3 α_{2C} 6.9–7.4 antagonist at each	**8.2** antagonist	*K_i 47 M_3 (rat)*[12]	**8.1** inverse agonist	*Inactive (rat)*[10]	D_5 6.9 antagonist 5-HT_{1A} 6.2 antagonist 5-HT_{2C} 7.6–8.2 antagonist 5-HT_6 7.7–7.8 inverse agonist 5-HT_7 7.6 inverse agonist
Fluphenazine 	7.7 antagonist	**8.8** antagonist			*K_i 8.1 nM (rat)*[14]		7.7 antagonist	*K_i 340nM (rat)*[13]	7.5 antagonist	*Inactive (rat)*[10]	D_5 7.9 antagonist 5-HT_7 7.9 inverse agonist 5-HT_6 7.3–7.4 inverse agonist
Levomepromazine 	*K_i 54.3*[1]	*K_i* ***8.6***[1]	*K_i* ***8.3***[1]				*K_d 0.58nM antagonist*[2]				
Mirtazapine 					α_{2A} **7.7** antagonist	α_{2C} **7.7** antagonist	*pIC_{50} 9.6 inverse agonist*[6] ***8.98***		**7.2** antagonist		5-HT_{2C} **7.4** antagonist

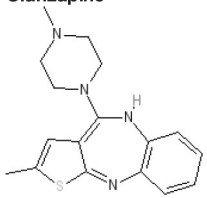
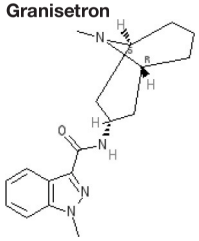
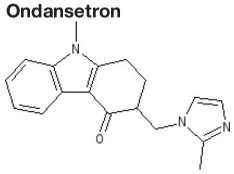
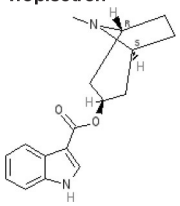
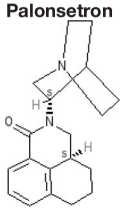
(Continued)

TABLE 2 | Continued

	D_1	D_2	D_3	D_4	α_1	α_2	H_1	M	5-HT_{2A}	5-HT_3A	Other
Metoclopramide											
Metoclopramide 		**7.5** antagonist (mouse)			*K_i >10,000 nM (rat)*		*K_i 1,100 nM (rat)*[13]	*K_i >10,000 nM (rat)*[13]		5-HT_3A **6.0–6.4** antagonist 5-HT_3AB **5.7** antagonist	5-HT_4 **6** agonist (mouse)
Butyrophenones											
Haloperidol 	7.6–8.2 antagonist	**7.4–8.8** antagonist	**7.5–8.6** antagonist	**8.7–8.8** antagonist	*K_i 46 nM*[5]	*K_i 360 nM*[5]	5.7–6.1 antagonist	*K_i >1,000 nM at M_1, M_2, M_3 (rat)*[5]	6.7–7.3 antagonist	*K_i >1,000 nM*[5]	5-HT_{1D} 6.6 antagonist 5-HT_7 6.3–6.6 antagonist 5-HT_{2B} 5.8–6.4 antagonist 5-HT_{1A} 5.7–5.8 antagonist
Droperidol 		*K_i 3 nM (rat)*[11]			*K_i 1.4 nM (rat)*[11]		*K_i 2,500 nM (rat)*[11]		*K_i 4.6 nM (rat)*[11]	*Inactive (rat)*[10]	
Domperidone 		**7.9–8.4** antagonist	**7.1–7.6** antagonist	*K_i 30.4 nM*[4]	*K_i α_{1A}, 71; α_{1B}, 530; α_{1D}, 710 nM*[9]					*Inactive (rat)*[10]	

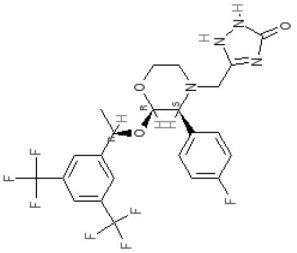
(Continued)

TABLE 2 | Continued

	D_1	D_2	D_3	D_4	α_1	α_2	H_1	M	5-HT_{2A}	5-HT_3A	Other
Second generation anti-psychotics											
Olanzapine 	*K_i 31 nM (rat)*[5]	**8.7** antagonist		*K_i 27 nM (rat)*[5]	*α_{1A} K_i 115 nM*[12]	*α_{2A} Ki 314; α_{2B} 81.6; α_{2C} 28.8 nM*[12]	**8.7–9.2** antagonist	*K_i 105 nM at M_3*[12]	**8.6–8.9** antagonist	*K_i 57 nM (rat)*[5]	5-HT_{2C} **8.1–8.4** inverse agonist 5-HT_6 **8** inverse agonist 5-HT_7 6.5 antagonist
5-HT_3 Receptor Antagonists											
Granisetron 		*Inactive (>10,000 nM) (rat)*[10]								5-HT_3A **~8.6–8.8** antagonist	
Ondansetron 										5-HT_3A **~7.8–8.3** 5-HT_3AB **7.8** antagonist	
Tropisetron 		*Inactive (>10,000 nM) (rat)*[10]								5-HT_3A **8.5–8.8** antagonist	5-HT_4 6.3–7.1 antagonist
Palonsetron 										5-HT_3A **10.5** antagonist	

(Continued)

TABLE 2 | Continued

	D_1	D_2	D_3	D_4	α_1	α_2	H_1	M	$5\text{-}HT_{2A}$	$5\text{-}HT_3A$	Other
NK_1 receptor antagonists											
Aprepitant 											NK_1 **10.1** antagonist

Data are from http://www.guidetopharmacology.org/ and are given as pK_i-values for the human receptors (usually recombinant); primary affinities are in bold. Additional information on drug affinity for human receptors (unless otherwise specified) is provided in italics together with references, listed below. Little information is known for cyclizine or for cinnarizine, particularly in terms of their interactions with human receptors and mechanisms. Clarke and Dundee (1971) describe significant sedation/drowsiness associated with promethazine, thiethylperazine, cyclizine and hyoscine. [1]*Srivastava et al., 2009;* [2]*Kanba and Richelson, 1984;* [3]*Meltzer et al., 1989;* [4]*Moreland et al., 2004;* [5]*Bymaster et al., 1996;* [6]*Bakker et al., 2007;* [7]*Liu et al., 2006;* [8]*Appl et al., 2012;* [9]*Kaiser et al., 2009;* [10]*Hamik and Peroutka, 1989;* [11]*Peroutka and Synder, 1980;* [12]*Kroeze et al., 2003;* [13]*Peroutka and Snyder, 1982;* [14]*Ison and Peroutka, 1986;* [15]*Norton et al., 1954;* [16]*https://www.drugbank.ca/drugs/DB00568#BE0000442.*

1947). These studies were rapidly followed by demonstration of the anti-emetic efficacy of hyoscine among soldiers in assault craft during tropical conditions (Hill and Guest, 1945). Today, drugs such as scopolamine are widely available for the treatment of all causes of motion sickness, manufactured by different companies in oral formulations and in more convenient formulations for anyone already experiencing nausea, such as transdermal patches and nasal sprays (Spinks and Wasiak, 2011; Golding and Gresty, 2015). Following identification of the different human muscarinic receptor subtypes (Huang et al., 2001) these drugs have been shown to act most notably at the M_3 and M_5 receptors which mediate cholinergic activity within the vestibular input to the vestibular nuclei and probably also within brainstem pathways integrating vomiting such as the NTS (Golding and Stott, 1997; Soto and Vega, 2010)

Antihistamines

The discovery of the "antihistamines" (the term histamine receptor antagonist was not introduced until 1966; Ash and Schild, 1966) was initiated by academic curiosity in 1937 (at the time compounds were known to block the actions of adrenaline and acetylcholine, so why not histamine?) and then rapidly further developed by the pharmaceutical industry. Initial success was achieved by Rhône-Poulenc Laboratories (**Tables 1**, **2**) screening "libraries" of compounds previously synthesized during a search for therapeutic alternatives to the anti-malaria drug quinine (from compounds traditionally used in the dying industry but known to exert anti-septic, anti-helminthic and anti-malarial activity), the supply of which was hindered by blockades imposed on Germany during WWI and then in WWII by Japanese expansion into South-East Asia (López-Muñoz et al., 2005). The first antihistamine to treat anaphylaxis and allergic reactions was phenbenzamine (also known as antergan), introduced into the clinic by Rhône-Poulenc in 1942. This was followed by diphenhydramine, chlorpheniramine, brompheniramine, promethazine and cyclizine (Emanuel, 1999; Sneader, 2005; Church and Church, 2013). Notably, H_1 receptor antagonism also supresses a number of different pathways within the brain, including those involved in arousal, leading to drowsiness, somnolence and sedation. As a counter-measure, dimenhydrinate (Dramamine) was introduced by G.D. Searle & Co, consisting of diphenhydramine with 8-chlorotheophylline (a mild stimulant and derivative of theophylline). Later, in the 1980s, other compounds were identified with poor ability to cross the blood brain barrier, the so-called "second generation" H_1 receptor antagonists, which do not have anti-emetic activity (Slater et al., 1999; Simons and Simons, 2011).

The discovery of antiemetic activity among the first generation antihistamine drugs was serendipitous. Dimenhydrinate (Dramamine) was undergoing evaluation in 1947 as a potential treatment of hay fever and urticaria. Among the patients receiving the drug was a pregnant woman who suffered from car sickness all her life. However, if she took dimenhydrinate a few minutes before boarding a tramcar she remained symptom-free; placebo was ineffective (Gay and Carliner, 1949). Next

year (1948) G.D. Searle & Co conducted a trial in which dimenhydrinate or placebo was given for 10 days or as a successful rescue therapy to 485 male USA troops crossing the Atlantic during "a rough passage" in the General Ballou, a converted freight ship (Gay and Carliner, 1949). In 1949 diphenhydramine itself (Benadryl) was shown to alleviate nausea and vomiting induced by streptomycin in four patients with pulmonary tuberculosis (Bignall and Crofton, 1949). These trials established the use of antihistaminic drugs as treatments of motion sickness and indicated that they may also be effective against emesis induced by other challenges. Cyclizine, developed in 1947 by Burroughs Wellcome, was shown to prevent sea- and air-sickness in 1952–1953 (see Norton et al., 1954 for references and data on the autonomic pharmacology of cyclizine) and has the notable history of being taken to the moon as a treatment for space sickness (**Figure 6**).

The first generation "antihistamines" (used to treat various allergic conditions; Simons and Simons, 2011) were effective against motion sickness, nausea and vomiting caused by labyrinthine disturbances (e.g., labyrinthitis and fenestration operations; Wang, 1965) and were investigated as anti-emetics in a number of other clinical settings (e.g., PONV, see Palazzo and Strunin, 1984; pregnancy, see Fairweather, 1978 and also Bhargava and Dixit, 1968, for pre-clinical studies). As anti-emetic drugs they are effective because they block H_1 receptors in the vestibular system and also in the brainstem integrative circuitry ("vomiting center") (Takatani et al., 1983; Soto and Vega, 2010). However, for some compounds, additional anti-emetic activity is thought to be due to their additional ability to antagonize at muscarinic receptors, perhaps not surprising, given the origin of the early compounds from a chemical template used to identify "adrenergic" and "cholinergic" antagonists (Liu et al., 2006). For example, in addition to antagonizing at the human H_1 receptor (Ki 12.6 nM), diphenhydramine also inhibits M_2 receptors (estimated Ki 80 nM) and displaces QNB binding in the cerebral cortex (Ki 280 nM; Kubo et al., 1987; Booth et al., 2002; Liu et al., 2006). Similarly, cyclizine and promethazine antagonize at the human H_1 receptor (respectively, Ki-values of 4.44 and 0.24 nM; Chazot et al., 2017) and appear to have an ability to inhibit the functions of acetylcholine (Norton et al., 1954). These drugs had no ability to prevent the vomiting initiated by apomorphine, a D_2 receptor agonist acting on the AP (see Carpenter et al., 1983 and also Borison and Wang 1953 and Borison, 1989 for review of evidence on the effect of area postrema ablation on the emetic response to apomorphine).

Histamine$_1$ receptor antagonists, in addition to illustrating how the pharmacological profile of compounds may change from that originally described, are also examples of a more fundamental shift in pharmacological characterization. Although the agents described above such as diphenhydramine are commonly referred to as H_1 receptor "antagonists," modern pharmacology now classifies them as "inverse agonists" (Bakker et al., 2007; Simons and Simons, 2011) but the implications of this for understanding both the mechanisms of emesis and the anti-emetic effects of different H_1 "antagonists" have not yet been considered (Tu et al., 2017).

Phenothiazines

The term "phenothiazines" refers to compounds with a nucleus of two benzene rings linked by a sulfur and a

FIGURE 6 | Photograph of the packaging for Marzine (cyclizine, developed in 1947) indicating its use by NASA during the Apollo moon missions. With permission: Wellcome collection, Wellcome Library (WF/M/PL/191), London, United Kingdom.

nitrogen atom to form a heterocyclic 3-ring compound, with phenothiazine itself first synthesized in 1883 (see Wang, 1965 for review). Chlorpromazine (Thorazine) was discovered from the observation that certain anti-histamines, in addition to prolonging sleep induced by barbiturates, also reduced the "shock" of surgery when given during anesthesia, somehow depressing the nervous system to leave patients relatively calm and relaxed during recovery. Re-examination of the antihistamines to optimize the "anti-shock" property (e.g., by testing for an increase in time required for trained rats to climb a vertical rope for food) led to synthesis of chlorpromazine (or 4560-R.P) in 1946 (Sneader, 2005). This compound had low antihistamine activity but blocked the effect of adrenaline on blood pressure and in research within SmithKline and French, inhibited conditioned reflexes in rats. The compound also prevented emesis evoked by apomorphine, acting on the AP in dogs (Glaviano and Wang, 1955). Apomorphine is primarily considered to be a D_2 receptor agonist but it is now more accurately defined as a potent agonist at the D_2 receptor subfamily (D_2, D_3, D_4) and D_5 receptors, with additional affinity for alpha$_1$- and alpha$_2$-adrenoceptors, 5-HT_{1A} and 5-HT_2 (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) receptors (Millan et al., 2002).

The pharmacological data on chlorpromazine, generated by Rhône-Poulenc in 1951, were published (Courvoisier et al., 1953) after the first clinical evaluation for treatment of "surgical shock" in 1952. The commercial name for chlorpromazine (Largactil) reflected its broad spectrum of activity ("large" = broad or wide, "acti" = activity) (López-Muñoz et al., 2005). Later, Carlsson (Nobel Prize Winner) and Lindqvist (1963) showed that chlorpromazine binds to postsynaptic dopamine receptors, launching the "dopamine hypothesis of schizophrenia" (in which symptoms could be treated by blocking dopamine receptors in post-synaptic neurons; Snyder et al., 1974) and revolutionizing treatment of psychiatric disorders.

Chlorpromazine was originally used to treat "neurosis" (sedation in psychiatric patients) and as pre-anesthetic medication, inhibiting nausea and vomiting, "shock" and augmenting the effects of anesthetics (Moyer et al., 1955). The anti-emetic activity of chlorpromazine was evaluated in more detail by Boyd et al. (1953, 1954) using dogs and apomorphine. This work was initiated after Prof. R. Paul (Faculté libre des Sciences d'Angers) visited his laboratory in November 1951, during which he described experiments at Rhone-Poulenc, as yet unpublished, showing 4560-R.P potentiating the action of sedatives and inhibiting apomorphine-induced vomiting in dogs. Prof. Paul arranged to have some sent to his laboratory, so its anti-emetic activity could be compared with promethazine, a structurally-related compound the authors had previously reported to have limited anti-emetic activity. The results clearly demonstrated the ability of chlorpromazine to prevent apomorphine-induced emesis in dogs. Contemporaneously, Brand et al. (1954) reported similar findings in dogs, using apomorphine, morphine and ergot as the emetic stimuli, but failed to prevent emesis evoked by copper sulfate or inhibit the response to any emetic stimulus in cats. In addition, the structurally related antihistamine, promethazine (Phenergan) had no ability to inhibit apomorphine-induced emesis. These data were consistent with Schmidt et al. (1953) who used dimenhydrinate and diphenhydramine. Later, a more detailed comparison using a number of phenothiazines (chloropromazine, promazine, trifluoperazine, levomepromazine, prochlorpromazine), trimethoxybenzamide, antihistamines (perphenazine, thiethylperazine, dimenhydrate, cyclizine), and hyoscine (Wyant, 1962) confirmed and extended these observations in dogs, demonstrating the ability of the phenothiazines and trimethoxybenzamide to prevent apomorphine-induce vomiting but to have lower activity against emesis evoked by intra-gastric copper sulfate, whereas the reverse was demonstrated by the antihistamines and by atropine.

These data were interpreted by reference to a series of experiments into the mechanisms and pharmacology of vomiting, reviewed by Borison and Wang (1953). The authors determined that vomiting induced by apomorphine (primarily a D_2 receptor agonist; see earlier) was caused by direct stimulation of the AP, considered the site at which emetic substances in the blood could induce emesis. Thus, chlorpromazine and the other phenothiazine derivatives acted primarily by blocking dopamine receptors (the term D_2 receptor was introduced by Kebabian and Calne, 1979) and although previously suggested, it was not until 1981 that the presence of D_2 receptors within the AP of dogs was confirmed (Stefanini and Clement-Cormier, 1981). The drugs also exerted some general sedative effects, but failed to prevent emesis induced by intra-gastric copper sulfate via visceral afferent activation.

Chlorpromazine was first evaluated as an anti-emetic in humans by cautious administration to patients with terminal cancer or uremia and then, following success, it was given to patients with a range of disorders, including labyrinthitis, psychological vomiting and pregnancy sickness, in addition to patients suffering from vomiting induced by a variety of drugs (Friend and Cummins, 1953, 1954).

Wampler (1983) provides the structures of the different phenothiazines and discusses their relative efficacies and adverse events. In summary, there is little evidence for differences in anti-emetic activity but differences in "anti-adrenergic," "anti-histaminic," and "anti-serotonin" activities confer variations in side-effects of sedation and hypotension. The strong "anti-adrenergic" activity of chlorpromazine, for example, was associated with hypotensive side-effects. Today, chlorpromazine has been shown to have approximately similar affinity for human H_1, α-adrenoceptor$_{2B}$, D_2, D_3 and 5-HT_{2C} receptors (acting as an antagonist) and for 5-HT_{2A} and D_5 receptors, acting as an inverse agonist[4] Examples of piperazine side-chain phenothiazines that have potent antiemetic activity include perphenazine, prochlorperazine and thiethylperazine maleate. These drugs (particularly prochlorperazine) were rapidly adopted for clinical use in a number of settings including anti-cancer chemotherapy, later becoming the comparator for newer agents (e.g., metoclopramide, cannabinoids; see Harris and Cantwell, 1986).

[4]http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?tab=biology&ligandId=83

Metoclopramide

This drug was identified by Laboratoires Delagrange in France in the mid-1950s, during a programme aimed at improving the properties of procainamide, a cardiac anti-arrhythmic and local anesthetic drug derived from procaine. Although some anti-emetic activity was known to exist within this class of molecule, chlorination of the benzene ring of procainamide (2-chloroprocainamide) significantly increased anti-emetic activity in dogs. However, more interesting was the absence of the sedative activity of the phenothiazine structures prompting an evaluation of related structures. In particular, methoxy-2-chloro-5-procainamide or metoclopramide, had negligible local anesthetic or cardiac anti-arrhythmic activity but an ability to inhibit emesis in dogs evoked by apomorphine and hydergine, in addition to copper sulfate (Justin-Besancon et al., 1964). Soon after, metoclopramide was found to stimulate gastric emptying, speed the rate of transit through the small intestine and reduce symptoms associated with various upper digestive tract disorders (Boisson and Albot, 1966; Robinson, 1973; Schulze-Delrieu, 1979; Gralla, 1983; Sanger and King, 1988). Between 1967 and 1971 several clinical trials evaluated the ability of metoclopramide to inhibit emesis, mostly in patients experiencing PONV, with perphenazine, trimethobenzamide, prochlorperazine and perphenazine as the comparators (Robinson, 1973). Delagrange undertook limited marketing of metoclopramide, also licensing to A. H. Robins (later acquired by American Home Products, which changed its name to Wyeth) for the USA markets, and with some initial skepticism over its wide range of potential clinical usage (Robinson, 1973), to Beecham Pharmaceuticals in the UK.

As dopamine receptors were characterized (Kebabian and Calne, 1979), metoclopramide was shown to be a D_2 receptor antagonist, selective over the D_3 receptor and the α_1-adrenoceptor (Rosenfeld et al., 1982; Andrews and Sanger, 2014). The drug found widespread use as an anti-emetic (e.g., during post-operative care or for patients with gastritis, migraine, dysmenorrhea and drug- or treatment-induced forms of emesis including that caused by anesthesia, radiation and some anti-cancer chemotherapies) and as a stimulant of upper gut motility (e.g., patients with gastro-esophageal reflux disease, gastroparesis, and functional dyspepsia; Pinder et al., 1976; Harrington et al., 1983). Initially, both the anti-emetic and prokinetic activities were attributed to dopamine receptor antagonism (**Table 3**). Although a major drug (there are now many generic versions across the world), its limited central action as an anti-emetic is, nevertheless, illustrated by its relative ineffectiveness in motion sickness. Further, at conventional doses (20 mg × 3 orally), the drug showed little or no anti-emetic superiority over placebo or prochlorperazine, when evaluated against the highly emetogenic agent cisplatin (e.g., Moertel and Reitemeier 1969), a relatively new anti-cancer drug at the time.

During the 1980s it was discovered that metoclopramide possessed an additional ability to stimulate gastric motility by activating 5-HT_4 receptors and at higher concentrations than those required to antagonize at the D_2 receptor, acting as a 5-HT_3 receptor antagonist (Sanger, 2009; see below). The former provided the mechanism by which metoclopramide stimulated GI motility and the latter heralded the development of new anti-emetic treatments and a revolution in care of cancer patients. These developments occurred during a time when 5-HT receptor pharmacology was being redefined.

The classification of 5-HT receptors began in 1957 when using guinea-pig ileum as their model, Gaddum and Picarelli defined a 5-HT M receptor (neuronally-mediated muscle contractions, blocked by morphine and also by atropine, cocaine, and methadone, even after dibenzyline) and a 5-HT D receptor (non-neuronally-mediated smooth muscle contractions, blocked by dibenzyline and also by lysergic acid diethylamide, dihydroergotamine, and 5-benzyloxygramine, even after morphine; Gaddum and Picarelli, 1957). In 1986 the classification was updated and three receptors defined: 5-HT_2 (5-HT D), 5-HT_3 (5-HT M) and a tentative "5-HT_{1-like}" receptor, with similarities to a heterogeneous group of 5-HT_1 (high affinity) binding sites (Bradley et al., 1986). Today, seven different 5-HT receptors have been cloned and characterized, with subtypes for some of these. All are GPCRs except 5-HT_3, a ligand-gated cation channel with potentially heterogeneous subunits (5-HT_3A-E; Holbrook et al., 2009).

In the 1980s a growing understanding of the mechanisms of action of metoclopramide became a significant factor in the discovery of the 5-HT_4 receptor. Firstly, it became clear that D_2 receptor antagonism could not fully explain how metoclopramide increased GI motility; for example, the more selective D_2 receptor antagonist domperidone did not mimic the ability of metoclopramide to facilitate cholinergic activity in human isolated stomach, thought to model the cholinergic-mediated gastric prokinetic activity of this drug (Sanger, 1985a). Thus, it was argued that metoclopramide acted on cholinergic nerves within the enteric nervous system (ENS), but not necessarily on other cholinergic neurons outside the ENS. Clearly, this activity in human isolated stomach was independent of brain function, consistent with the inability of vagotomy to prevent the prokinetic effects of metoclopramide (Jacoby and Brodie 1967). These and other experiments demonstrated that metoclopramide facilitated ongoing cholinergic activity, increasing the release of acetylcholine (ACh) rather than directly stimulating muscarinic receptors (Sanger, 2017). This activity was not due to antagonism at pre-junctional muscarinic receptors, was not blocked by antagonists at the adrenoceptors or D_2 receptors, or by antagonists at various other receptors and mechanisms. Instead, relatively high concentrations of 5-HT mimicked the response and non-selective ligands for 5-HT receptors mimicked or blocked this action of metoclopramide (Sanger, 1985b,c, 1987a); the notable exception was the failure to mimic or inhibit with a 5-HT_3 receptor antagonist, leading to the proposal that metoclopramide and related compounds such as renzapride, facilitated cholinergic activity within the ENS by activating a "myenteric 5-HT-like receptor" (Sanger, 1987a,b). This was quickly linked to a "non-classical" 5-HT receptor identified by Dumuis et al. (1998) in mouse embryo colliculi neurons and in guinea pig hippocampal membranes and later defined as the 5-HT_4 receptor (Bockaert et al., 1992).

TABLE 3 | Changing understanding of the role of gastric motility in the genesis of nausea and vomiting: Influences on drug discovery.

	CONCEPT: Gastric prokinetics help patients with delayed gastric emptying including functional dyspepsia/gastroparesis (multiple symptoms, including nausea, vomiting, early satiety)	
Mid-1950s Metoclopramide synthesized[1]	**5-HT_4 receptor agonists** Metoclopramide • 5-HT_4 agonist, D_2 antagonist (later shown to be a 5-HT_3 antagonist)[2] • Used in GERD, functional dyspepsia, gastroparesis; the only prescribed drug for gastroparesis in the USA[3]	**CURRENT STATUS:** 1. Gastric Prokinetics (5-HT_4 and motilin agonists) useful in patients requiring more rapid delivery of (for example) orally administered drugs to the intestine[17] 2. No consistent correlation between symptoms (e.g., nausea, early satiety) and delayed gastric emptying[18] 3. Gastric prokinetic and direct anti-emetic activity of metoclopramide confuses mechanism of therapeutic action 4. Role of erythromycin in the treatment of gastroparesis uncertain
Other substituted benzamides (eventually shown to be 5-HT_4 receptor agonists)	Cisapride • 5-HT_4 agonist, poor D_2 antagonist (later shown to have similar affinity for 5-HT_{2A}, 5-HT_{2B}, α_1-adrenoceptors and low affinity for 5-HT_3)[4,5] • 5-HT_{2A} and 5-HT_{2B} also implicated in mechanisms of emesis[6] • Reduced nausea in certain patients (now withdrawn)[7,8]	
Explored	Others • Some animal data suggests ability to inhibit vomiting but non-selectivity of action makes it difficult to interpret[9] • Gastroprokinetic activity may oppose ability of 5-HT_3 antagonists to inhibit severe emesis in ferrets[10]	
1989 Erythromycin proposed to act as a motilin receptor agonist[11]	**Motilin receptor agonists** Erythromycin • Antibiotic drug used at lower doses to treat patients with gastroparesis and delayed gastric emptying[12] • Activates motilin receptors in enteric nervous system (prokinetic activity) and vagus[13,14,15] • Low doses may have anti-emetic activity; high doses cause emesis[15] • Limited by potential to exacerbate bacterial resistance, prolong cardiac QTc interval, and interact with cytochrome P450 CYP 3A4[12] • The selective motilin agonist camicinal shown to promote gastric emptying and facilitate oral drug delivery in patients with Parkinson's disease[16]	
	CONCEPT: Selective dopamine D_2 antagonists are anti-emetic but also increase gastric emptying, making them additionally useful treatments of gastroparesis (as defined by delayed gastric emptying)	
1974 Domperidone synthesized[19]	Domperidone Increased gastric emptying in gastroparesis[20] Alleviates symptoms of gastroparesis[21] No effects on gastric emptying in healthy volunteers[22] or in patients requiring video capsule delivery to the small intestine[23] and no direct ability to influence contractility of human isolated stomach[24] Low risk of cardiac QTc prolongation[25] Registered for use in many countries but not in the USA[21]	**CURRENT STATUS:** 1. Domperidone still explored in treatment of gastroparesis[21] 2. Selective 5-HT_3[25] and NK_1 antagonists[27] have anti-emetic effects but do not increase gastric emptying although they may have benefits in patients with gastroparesis. 3. These data support a role for dopamine in regulation of gastric motility in addition to emesis during disease
	CONCEPT: Ghrelin agonists increase gastric emptying, leading to exploration of their potential to treat gastroparesis, enhanced by ability to promote appetite/reduce emesis	
1999 Ghrelin discovered and sequenced[28]	• Increase gastric emptying in healthy volunteers and in patients with gastroparesis but may not be sustained with long-term dosing[29] • No direct ability to influence contractility of human isolated stomach[30] • Increases appetite and reduced nausea in patients, including gastroparesis[31]	**CURRENT STATUS:** 1. Ghrelin agonists remain of interest because they can reduce nausea and increase appetite[29]
	CONCEPT: Dysrhythmic movements of the stomach cause nausea and/or are the result of nausea	
2017 Resurgence of interest in the relationships between gastric dysrhythmia, gastric emptying, nausea and vomiting and gastric pathology in patient sub-groups	Gastric Dysrhythmia • Association between nausea and dysrhythmia of gastric myoelectric activity characterized using electrogastrography in several groups of patients, strengthened by studies with dense recording arrays[32,33] Interstitial Cells of Cajal (ICC) • Responsible for electrical slow waves; damage associated with gastric dysrhythmia (initiation, propagation)[34] • Hypothesis: Nausea caused by vagal afferents detecting gastric dysrhythmia and signaling to brain stem[32,35]	**CURRENT STATUS:** 1. Exploratory research of ICCs as drug targets 2. Improved clinical classification of patient groups with delayed gastric emptying

[1]*Justin-Besancon and Laville, 1964;* [2]*Sanger, 2009;* [3]*Camilleri et al., 2013;* [4]*Briejer et al., 1995;* [5]*Smith et al., 2008;* [6]*Johnston et al., 2014;* [7]*Creytens, 1984;* [8]*Bergeron and Blier, 1994;* [9]*Sanger et al., 2013;* [10]*Miner et al., 1987;* [11]*Peeters et al., 1989;* [12]*Sanger et al., 2013;* [13]*Broad et al., 2012;* [14]*Broad and Sanger, 2013;* [15]*Javid et al., 2013;* [16]*Marrinan et al., 2018;* [17]*Sanger and Alpers, 2008;* [18] *Janssen et al., 2013;* [19]*Champion et al., 1986;* [20]*Ahmad et al., 2006;* [21] *Heckert and Parkman, 2018;* [22]*Markey and Shafat, 2012;* [23]*McFarlane et al., 2018;* [24]*Sanger, 1985c;* [25]*Ortiz et al., 2015;* [26]*Midani and Parkman, 2016;* [27]*Pasricha et al., 2016;* [28]*Kojima et al., 1999;* [29]*Sanger and Furness, 2016;* [30]*Broad et al., 2014;* [31]*Camilleri and Acosta, 2015;* [32]*Koch, 2014;* [33]*O'Grady et al., 2012;* [34]*Angeli et al., 2015;* [35]*Sanger and Pasricha, 2017.*

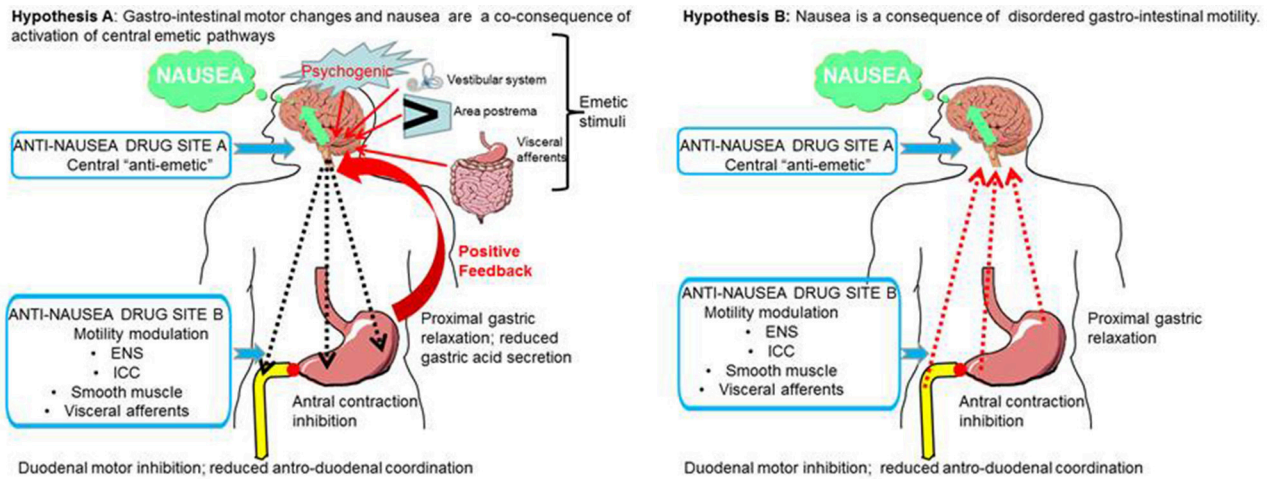


FIGURE 7 | Two hypotheses for the relationship between disordered upper gastrointestinal tract motility and the sensation of nausea. These are not mutually exclusive but the efficacy of drugs targeted at sites **A** and **B** will differ depending upon which mechanism is in operation. In **hypothesis A** (left hand panel) the activation of central emetic pathways activates ascending pathways leading to the sensation of nausea, followed by descending autonomic pathways leading to delayed gastric emptying. An anti-nausea drug targeted centrally (site **A**) would block both nausea and the peripheral motility changes, so there will be a secondary return of gastric emptying to normal. In this hypothesis a drug targeted at site **B** may only have a small effect by reducing a positive reinforcing feedback from the centrally-driven disruption of motility. In **hypothesis B** (right hand panel) disordered upper digestive tract motility, usually resulting from disease (e.g., diabetic gastroparesis), is the primary driver for the genesis of nausea, leading to activation of visceral afferents or possibly the release of enteroendocrine agents into the blood for subsequent activity at the area postrema. A drug acting on the upper digestive tract (site **B**) would normalize gastric motility and remove the primary drive for nausea. Note that in this hypothesis, the "traditional prokinetic drugs" (with an exclusively peripheral action) have generally not been successful; potential alternatives are indicated. In this hypothesis a drug acting at the central site **A** would also be likely to indirectly reduce nausea by preventing activation of central pathways. ENS, enteric nervous system; ICC, interstitial cells of Cajal.

Domperidone

Among the antipsychotic compounds (including the butyrophenone haloperidol, discovered in 1958 by Paul Janssen; Sneader, 2005) Janssen Pharmaceutica (**Tables 1**, **2**) developed in the mid-1950s, some were effective antagonists at the dopamine receptors in the AP involved in induction of vomiting. Since this region of the brain has a relatively permeable blood-brain barrier, a search was made for antagonists that did not cross this barrier and hence, were less likely to evoke extrapyramidal side-effects caused by antagonism of dopamine receptors within the brain. Using the now-established model of apomorphine-induced emesis in dogs, domperidone was identified in 1974 from the butyrophenone class of molecules. The drug was erroneously described as similar to metoclopramide (Champion et al., 1986; perpetuating the belief that all of the actions of metoclopramide must be due to antagonism of the effects of dopamine) and marketed in 1982 (Champion et al., 1986; Barone, 1999) for prevention of nausea and vomiting (**Figure 7**) including that induced by anti-cancer chemotherapy, then as a gastroprokinetic agent (Ahmad et al., 2006) and galactogogue. Later studies showed that domperidone has a similar affinity for the human D_2 and D_3 receptors (Ki-values, respectively, 12.6 and 4 nM[5]), no ability to interact with the 5-HT_4 receptor but at slightly higher concentrations acts as a α_1-adrenoceptor antagonist (Ki of 71 nM: Keiser et al., 2009; see also Ennis and Cox 1980; Ison and Peroutka, 1986).

Investigation of the utility of dopamine receptor antagonists as anti-emetics continues with investigations of other D_2/D_3 receptor antagonists, such as amisulpride (Kranke et al., 2013) and ATC-1906[6], aiming primarily to achieve an improved safety profile over domperidone (i.e., its cytochrome P450 interaction liability and occasional reports of prolongation of cardiac QTc intervals; Ortiz et al., 2015) and gain access to patients in the USA (where domperidone is not registered) as well as the rest of the world, for treatment of gastroparesis.

Dexamethasone; A Synthetic Glucocorticoid

Baker et al. (1979) found that dexamethasone (10 mg) reduced vomiting caused by different cytotoxic anti-cancer drugs but it was suggested that the associated euphoria played a role. A pilot study using methylprednisolone to inhibit prostaglandin release (Rich et al., 1980) also showed efficacy (in combination with chlorpromazine or prochlorperazine) in patients receiving cisplatin-based therapy. Later studies using high-dose dexamethasone in patients receiving cisplatin alone or in combination with other cytotoxic drugs reported impressive responses with excellent or good control of nausea and vomiting in 50% of patients who had failed on standard anti-emetics and

[5]http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?tab=biology&ligandId=965

[6]https://www.pharmpro.com/news/2016/07/takeda-altos-therapeutics-partner-develop-gastroparesis-treatment

71% in patients who had not received anti-emetics previously (Aapro and Alberts, 1981). Notably, synthetic corticosteroids do not inhibit the acute, rapid-onset forms of vomiting induced by apomorphine or ipecacuanha (Axelsson et al., 2003; Sam et al., 2003), suggesting involvement of an "inflammatory" component in the mechanisms of chemotherapy-induced emesis (Sanger and Andrews, 2006).

Although now widely used in combination with other anti-emetic drugs the mechanism and site of action is still not clear. One suggestion is that dexamethasone may supress eicosanoid metabolism, inflammation and edema induced by chemo-radiotherapy (Andrews and Rudd, 2016; see Chu et al., 2014 for review).

Cannabinoids

In the early 1970s, anecdotal reports emerged of reduced nausea and vomiting by marijuana-users undergoing chemotherapy for Hodgkin's disease, leading to clinical evaluation of the anti-emetic use of marijuana and THC (Δ-9-tetrahydrocannabinol, the major psychoactive constituent) in cancer patients receiving chemotherapy (Sallen et al., 1975; Vincent et al., 1983; Parker et al., 2011). Thereafter, the Food and Drug Administration (FDA) was recommended by the Oncologic Drug Advisory Committee to classify THC for use against refractory chemotherapy-induced emesis (Vincent et al., 1983). Cannabinoids (THC, nabilone, levonantrodol) were extensively investigated as anti-emetics in anti-cancer chemotherapy in the late 1970s and early 1980s with a 1981 survey indicating THC inclusion in 26.5% of studies, intermediate between prochlorperazine (41.2%) and metoclopramide (20.6%; Penta et al., 1981). Although cannabinoids were superior to placebo and prochlorperazine, they were not pursued at the time because of side-effects and probably also because of the discovery of the anti-emetic efficacy of 5-HT_3 receptor antagonists a few years later (see below).

Developments in cannabinoid receptor pharmacology and the availability of selective ligands prompted a resurgence of interest in the anti-emetic effects of cannabinoids (Darmani, 2001; Simoneau et al., 2001) and particularly their potential in treatment of chemotherapy-induced nausea (Rock and Parker, 2016). These agents have been shown to be effective against vomiting and behaviors suggestive of nausea (see below) in several animal models (ferret, least shrew, house musk shrew, rat). In contrast to other agents discussed above, they act as a receptor agonist, activating CB_1 receptors in the dorsal vagal complex of the brainstem (Van Sickle et al., 2003) and the visceral insular cortex (Limebeer et al., 2012). The clinical potential of the selective CB_1 receptor agonists remains to be evaluated.

THE 1980s: A NEW ERA IN CONTROL OF NAUSEA AND VOMITING PROMPTED BY CHANGES IN CHEMOTHERAPY

The rise in treatment of cancer from the 1960s to 1980 also saw an increase in the number of anti-emetic studies in cancer patients. From 1963 such studies increased from 1 to 12 *per annum* in 1980 involving 25 different compounds alone or in combination (Penta et al., 1981). An important driver was the introduction of more effective, but unfortunately more emetic, chemotherapy agents and in particular cisplatin, in 1971 (for history of platinum salts, see Christie and Tansey, 2007). The limited efficacy of anti-emetic drugs in these new therapeutic regimes prompted research which led to the discovery of 5-HT_3 receptor antagonists, discussed below. A clinical study published in 1984 (Plezia et al., 1984) reported that acute vomiting induced by cisplatin-containing treatments could be blocked by an "intensive five drug regime" (metoclopramide, diphenhydramine, dexamethasone, diazepam, thiethylperazine); by 1988 it was possible to achieve the same effect by intravenous injection of a 5-HT_3 receptor antagonist alone (Cassidy et al., 1988). Although the introduction of cisplatin was a significant stimulus for research into novel anti-emetic drugs it should not be forgotten that radiation was also used to treat cancer and also given prior to bone marrow transplantation, causing severe nausea and vomiting (Danjoux et al., 1979); as late as 1978 general anesthesia was being used to prevent acute vomiting resulting from total body irradiation (Whitwam et al., 1978).

5-Hydroxytryptamine$_3$ Receptor Antagonists

Gylys et al. (1979) found that in conscious dogs, metoclopramide more effectively inhibited vomiting evoked by cisplatin, compared with chlorpromazine, haloperidol, domperidone, or nabilone. Then in 1981, high intravenous doses of metoclopramide were shown to reduce emesis in patients receiving cisplatin for treatment of cancer, contrasting with the poor effectiveness of prochlorperazine (Gralla et al., 1981). The rationale for using the high dose was later explained by Gralla (Christie and Tansey 2007). In brief, they realized that the phenothiazines and the cannabinoids were not working well so they needed another approach. In the USA, metoclopramide was still a relatively new drug (it was widely used in Europe) and since the dose was not well-established for the indication of emesis it was decided to undertake a trial that escalated the dose to maximize the chance of success. As Gralla recalled "I looked at the world's suicide literature and it looked as though it was impossible to kill yourself with the drug, so that sounded good." Following the successful use of high-dose metoclopramide later trials failed to replicate this activity with high doses of the D_2 receptor antagonists domperidone (no change in protection but serious side-effects noted; Tonato et al., 1985) and alizapride (less effective than metoclopramide and caused severe hypotension; Saller and Hellenbrecht, 1985). Thus, it began to seem unlikely that high doses of metoclopramide achieved greater anti-emetic activity simply because it somehow blocked D_2 receptors in the brain more effectively. At that time, one possibility was that the ability of metoclopramide to increase gastric emptying may in some way supplement the anti-emetic activity of this drug by accelerating emptying of the stomach thus overcoming the gastric stasis which accompanies nausea and precedes vomiting (see **Figure 7**).

The anti-emetic activity of metoclopramide was confirmed by use of a ferret model of emesis to demonstrate efficacy against different chemotherapeutic agents. The model was introduced by Floczyk et al. (1982) using cisplatin as the emetic stimulus, confirmed by Miner and Sanger (1986) and quickly extended to study the effects of the chemotherapeutic drugs doxorubicin and cyclophosphamide (Schurig et al., 1984; Miner et al., 1987) and whole body irradiation (Gylys and Gidda 1986; Andrews and Hawthorn 1987; Miner et al. 1987). The history of the use of the ferret in anti-emetic research is reviewed Percie du Sert and Andrews (2014) and this model has largely supplanted the use of dogs as the first species in which novel anti-emetics are studied and as a species for investigating emetic potential of NCEs.

Research within Beecham Pharmaceuticals (**Figure 2**; **Table 1**) using ferrets showed that cisplatin-induced emesis was unaffected by domperidone but prevented by renzapride (BRL24924), a molecule originally identified as a potent stimulant of gastric motility (and an agonist at the "myenteric-like 5-HT receptor" or 5-HT_4; see above) without ability to antagonize at the D_2 receptor (and subsequently shown to potently antagonize at the 5-HT M or 5-HT_3 receptor; Miner et al., 1986, 1987; Sanger, 1987a). Since these experiments could not rule out the possibility that anti-emetic activity was achieved by stimulation of gastric emptying alone (Alphin et al., 1986) it was necessary to perform additional experiments with the recently described selective 5-HT_3 receptor antagonist MDL72222 (a generous gift to G.J. Sanger from J.R. Fozard, then at Merrel-Dow). The resultant complete control of vomiting demonstrated for the first time, that powerful anti-emetic activity could be achieved by 5-HT_3 receptor antagonism alone (Miner et al., 1986).

Prior to these studies in ferrets it had become clear that metoclopramide could also interact with 5-HT receptors which were, at the time, poorly understood. The drug antagonized a neuronally-mediated action of 5-HT in guinea-pig isolated colon and ileum (Bianchi et al., 1970; Birtley and Baines, 1973; Bury and Mashford, 1976; Fozard and Mobarok Ali, 1978), defining metoclopramide as a 5-HT M receptor antagonist. Metoclopramide could also antagonize other neuronally-mediated actions of 5-HT in the peripheral nervous system, most notably, 5-HT-evoked tachycardia in rabbit isolated heart or bradycardia in anesthetized rats (the von Bezold-Jarisch reflex; Fozard and Mobarok Ali, 1978; Fozard, 1983). Fozard and colleagues subsequently showed that (–)-cocaine and structurally-related compounds also antagonized these actions of 5-HT, leading to synthesis of MDL72222 from the chemical template of cocaine by Merrell Dow (**Figure 2**; **Tables 1**, **2**), the first selective 5-HT_3 receptor antagonist, then aimed at treatment of migraine (Fozard, 1984).

The anti-emetic experiments, conducted in the laboratories of Beecham Pharmaceuticals, were quickly replicated using their own compound (the selective 5-HT_3 receptor antagonist BRL43694 or granisetron; Boyle et al., 1987; Bermudez et al., 1988) and those from their competitors including: Glaxo (GR38032F or ondansetron, a racemate designed for "a variety of disorders including migraine" before being specifically patented for treatment of depression, schizophrenia, anxiety and cognitive disorders[7]); Sandoz (ICS 205-930 or tropisetron, designed for treatment of migraine and later found to have some ability to antagonize at the 5-HT_4 receptor); and Merrell Dow (MDL72222 or bemesetron, for treatment of migraine). These studies led to the filing of a patent claiming the use of these compounds for treatment of emesis Sanger and Miner 1988, successfully upheld over ondansetron (Cavella et al., 1997, p. 27). Significantly, anti-emetic efficacy was not just restricted to the control of cisplatin-induced-emesis but was equally effective against different chemotherapeutic drugs (Miner et al., 1987). Further, emesis could be controlled even after it had begun (Miner et al., 1987), later of great importance in positioning the 5-HT_3 receptor antagonists as both prophylactic treatments and for control of breakthrough emesis. An additional control experiment, required at the time, was to demonstrate that 5-HT_3 receptor antagonism by granisetron did not also prevent the anti-tumor activity of cisplatin (Goddard et al., 1990). There was now no doubt that the experiments within the Beecham Laboratories had demonstrated the role of the 5-HT_3 receptor in the mechanisms by which chemo- and radio-therapy evoke nausea and vomiting (reviewed in Sanger, 1990).

During this time and following the original abstract highlighting the anti-emetic activity of renzapride (Miner et al., 1986), experiments to demonstrate the anti-emetic activity of the 5-HT_3 receptor antagonist ICS 205-930 (Costall et al., 1986) were swiftly sponsored by Sandoz, the manufacturer of ICS 205-930 (see Christie and Tansey 2007). With respect to ondansetron and tropisetron, these can therefore be regarded as examples of "re-purposing" (bemesetron was not progressed for treatment of emesis, the company preferring its follow-up molecule MDL73147 or dolasetron; see Kirchner et al., 1993).

5-HT_3 receptor antagonists prevent cytotoxic-associated vomiting by blocking the ability of 5-HT, released from mucosal enterochromaffin cells in the upper GI tract, to activate 5-HT_3 receptors on abdominal vagal nerve terminals and thereby "desensitize" the vagus to the pro-emetic stimulatory actions of 5-HT and other substances (e.g., prostanoids) released during the cytotoxic treatment (Andrews et al., 1988; see Andrews and Rudd, 2016 for review of more recent evidence).

The more advanced stage of clinical and safety testing of ondansetron (for CNS disorders) meant that this drug was first to achieve registration by the FDA in 1991, followed in the same year by granisetron in other countries and in particular, by Japan in 1992. Later, there would be controversy over the number of published clinical trials reported for ondansetron, which appeared to have been reported more than once under different authorship in different publications (Rennie, 1999), calling for registration of clinical trials (now best practice). Nevertheless, today, selective 5-HT_3 receptor antagonists are an essential component of anti-emetic therapy in patients undergoing chemotherapy and together with the NK_1 receptor antagonists (see below) has revolutionized treatment of cancer and reduced health care costs (Currow et al., 1997; Warr and DeAngelis, 2009).

[7] https://www.google.co.ug/patents/US4973594

Neurokinin$_1$ (NK_1) Receptor Antagonists

The widespread clinical use of 5-HT_3 receptor antagonists to treat chemotherapy-induced nausea and vomiting (CINV) and to a lesser extent PONV, established the clinical need and hence, the market value of an anti-emetic drug, which could exceed one billion $US *per annum*, further stimulating interest in this therapeutic area. Additionally, the primary efficacy of 5-HT_3 receptor antagonists in the acute phase of highly emetic chemotherapy (e.g., cisplatin containing regimes) as compared to the delayed phase where they appeared less efficacious, their lower efficacy against nausea as compared to vomiting for both CINV and PONV, and their lack of effect against emesis induced by motion and apomorphine, illustrated the need for further developments.

Substance P was identified by von Euler and Gaddum in 1931; the name originates from the phrase in their paper "This standard preparation, which we call P…." (von Euler and Gaddum, 1931, p. 80). Over the last 40 years research into the actions of substance P has been most closely associated with pain pathways with focus on the neurokinin$_1$(NK_1) receptor as the primary receptor for substance P in mammals (see Borsook et al., 2012). Studies, largely in rodents, identified non-peptide small molecules acting as antagonists at the NK_1 receptor for potential clinical use as analgesics. During this time, the involvement of substance P (or other tachykinins) in mechanisms of nausea and vomiting was largely overlooked, despite a body of literature summarized in **Table 4**, which in many ways parallels that for its involvement in pain (see Andrews and Rudd, 2004). Definitive evidence for the involvement of substance P in emesis in animals came only with the development of the non-peptide, brain penetrant, NK_1 receptor antagonists disclosed by Pfizer (CP-96,435, Snider et al., 1991; CP-99,994, McLean et al., 1993). The first published studies showing anti-emetic effects were in the ferret by researchers at Glaxo (Bountra et al., 1993; Gardner et al., 1994) and Merck (Tattersall et al., 1993, 1994) but using a Pfizer compound (CP-99,994). These were followed by a detailed study in the ferret, cat, house musk shrew and dog from Pfizer with academic colleagues (Watson et al., 1995a,b). Overall the studies demonstrated that NK_1 receptor antagonists had a different profile from 5-HT_3 receptor antagonists (and muscarinic and H_1 receptor antagonists) in their ability to block both acute and delayed cisplatin-induced emesis, to block emesis induced by both peripherally (e.g., copper sulfate, abdominal vagal afferent electrical stimulation) and centrally-acting stimuli (e.g., morphine, apomorphine) and also to reduce motion-induced emesis. This unique preclinical profile rekindled interest in the area of anti-emetics. However, a major question was whether these encouraging pre-clinical findings (largely from the ferret) would translate to the clinic. This question arose because despite the pre-clinical data (largely from the rat) for the involvement of Substance P in pain pathways, contemporaneous published clinical studies of analgesic effects of NK_1 receptor antagonists were equivocal (e.g., Dionne et al., 1998; Reinhardt et al., 1998; see Rupniak and Kramer, 1999; Hill, 2000; Borsook et al., 2012 for reviews). Among the suggested reasons for this failure (Laird et al., 2000) was the potential for receptor/neurotransmitter redundancy in pain-conducting systems (e.g., for the NK_1, NK_2, NK_3 receptors small differences in affinity for endogenous ligands meant that "ligand promiscuity" was a real possibility; Maggi, 2000; Sanger, 2004) or a mismatch between the measure of "nociception" in animals and the human sensation of pain.

A key issue in increasing the likelihood that data obtained in the ferret would translate was the early recognition of marked species differences in NK_1 receptor pharmacology with some compounds having a relatively high affinity at the rat receptor compared the human NK_1 receptor (e.g., RP67580) whereas others had a relatively high affinity at the human compared to the rat receptor (e.g., L743310; see **Table 1**, p. 382, Andrews and Rudd 2004). Taking CP-99,994 as an example, as it was the compound most widely used in establishing the *in vivo* effects of NK_1 receptor antagonists, it has relatively high affinity at the human (K_i 0.3 nM) and ferret (K_i 1.7 nM) NK_1 receptors in contrast to the rat receptor (K_i 111 nM); a similar pattern is found with other NK_1 receptor antagonists (Andrews and Rudd 2004). *In vitro* autoradiographic studies showed that CP-99,994 displaced [^{3}H]-substance P from the ferret brainstem including the AP and the subnucleus gelatinosus region of the NTS in a concentration-related manner over 0.1–100 nM (Watson et al., 1995a). It should be noted that technological advances in brain imaging now make it possible to study ligand-receptor interactions *in vivo* in animals (e.g., Chin et al., 2006) and humans (e.g., Borsook et al., 2012) facilitating compound and clinical dose-selection and hopefully enhancing translation.

The first human study of an NK_1 receptor antagonist was published in 1997 (Kris et al., 1997), <4 years after the first pre-clinical publication. This rapid time was facilitated by prior safety studies required for the earlier analgesic studies (see above) and illustrates why progress can sometimes be rapid if a drug has already been investigated in another therapeutic area. In 17 patients undergoing highly emetogenic cisplatin chemotherapy CP-122,721 was efficacious overall but the effect was particularly marked (83% complete control) in the delayed phase of emesis. Further studies in patients undergoing chemotherapy followed, using other compounds (e.g., CJ-11,974, Hesketh et al., 1999; L-54030 and L758298, Navari et al., 1999) and compounds were also investigated for efficacy in PONV (CP-122, 721, Gesztesi et al., 1998; GR-205171, Diemunsch et al., 1999).

Currently, four NK_1 receptor antagonists are approved for human clinical use: aprepitant, fosaprepritant [intravenous formulation of aprepitant (see Hale et al., 1998, for characterization)], rolapitant, and netupitant, the primary differences being potency and duration of action. The most recent MASCC/ESMO guidelines for high emetic-risk chemotherapy (Herrstedt et al., 2017) recommend use of an NK_1 receptor antagonist in combination with a 5-HT_3 receptor antagonist and dexamethasone for optimal efficacy.

It is worthwhile noting that the NK_1 receptor antagonist maropitant (Benchaoui et al., 2007b) is marketed (Cerenia^{TM}) for prevention of acute vomiting in dogs. It has been used for treatment of vomiting in dogs undergoing cisplatin-chemotherapy (Vail et al., 2007) but also has efficacy against vomiting in other indications including parvoviral enteritis and

TABLE 4 | A summary of the key pieces of evidence implicating substance P and related tachykinins in emesis.

Date	Evidence	Species	References	Comment
1936	Substance P (SP) extracted from the vagus	Dog	von Euler, 1936	• The vagus had been implicated in the induction of emesis by early animal studies (Hatcher, 1924) and studied in the 1920s (cited in Lewis, 1942) when induction of nausea was reported in humans by stimulation of the vagus • Subsequent demonstration in the vagus and nodose ganglion of multiple species including human (e.g., Lundberg et al., 1978). Also vagal afferents terminating in the *nucleus tractus solitarius* (NTS) shown to be source of some of the Substance P in the dorsal brainstem (see Andrews and Rudd, 2004)
1951	High levels of SP extracted from the digestive tract mucosa	Dog	Douglas et al., 1951	• Digestive tract mucosa enterochromaffin cells shown to be a rich source of 5-HT in a range of species in the 1950s, accounting for the majority of 5-HT in the mammalian body (Faustini, 1955; Erspamer and Testini, 1959)
1954	High concentrations of SP in the area postrema (AP); Authors comment: "the AP only contains active substances by virtue of its chemoreceptive properties… One of the functions of some parts of this tissue may be to act as a chemoreceptor for substances in the blood stream to convert messages received in this way into nervous impulses."	Dog	Amin et al., 1954	• Although the AP was implicated in emesis by older papers (e.g., see Hatcher, 1924) the seminal paper by Wang and Borison (1952) highlighted its role as a chemoreceptive region of the brain
1963	Induction of emesis by subcutaneous administration of eledoisin (a tachykinin closely related to SP and extracted from posterior salivary glands of the octopod *Eledone cirrhosa*)	Dog	Erspamer and Glasser, 1963	• The frog skin tachykinin, pysalemin (subcutaneously and intravenously) induced vomiting in the dog Bertaccini et al. (1965) and intravenous SP was shown subsequently to have a similar effect, although so did many other peptides Carpenter et al. (1983, 1984)
1981	Immunohistochemical localization of SP in the AP to varicose processes but absence of SP-positive cell bodies	Rat	Armstrong et al., 1982	• Findings confirmed and extended by Pickel and Armstong (1984) but as rodents lack an emetic reflex (but see text for discussion) the relevance to emesis may have been overlooked. Newton et al. (1985) confirmed and extended the rat finding to the cat, a species with an emetic reflex so potentially of more relevance to humans.
1981	High levels of SP in human brainstem including area postrema	Human	Cooper et al., 1981	• A study in 1955 (i.e., a year after the (Amin et al., 1954) dog study) had found little SP in the human AP and may have led to the view that there were species differences, resulting in dismissal of the potential clinical significance of the dog study.
1983	Activation of AP neurons by ionophoretic application of SP	Dog	Carpenter et al., 1983, 1984	• Electrophysiological evidence for excitatory effects of SP in a relevant species, but numerous other peptides had similar effects, possibly reducing the significance of the observation
1984	Demonstration of high levels of SP receptors in the *nucleus tractus solitarius* and moderate levels in the area postrema	Rat	Helke et al., 1984	• SP-sensitive receptors investigated using [^{125}I]Bolton-Hunter SP • NTS implicated in coordination of visceral and somatic motor outputs for emesis and integration of afferent signals prior to projection to more rostral brain regions (see text for details)
1988	Induction of retching in the urethane anesthetized ferret by topical application of SP (0.1 mM) to the fourth ventricle	Ferret	Wood, 1988	• Proposed that the action was either directly on the AP or via access to the dorsal NTS, particularly the *subnucleus gelatinosus*. A subsequent study in conscious ferrets showed that injection of SP into the NTS induced emesis (Gardner et al., 1994)

(Continued)

TABLE 4 | Continued

Date	Evidence	Species	References	Comment
1992	Acute administration of the ultrapotent capsaicin analog (RTX) to the ferret has anti-emetic effects against both centrally and peripherally acting stimuli	Ferret	Bhandari and Andrews, 1992	• In the subsequent full paper (Andrews and Bhandhari, 1993) it was proposed that "the most likely mechanism to account for the anti-emetic effects is that RTX induces a depletion of a neurotransmitter, possibly substance P or CGRP, at a central site in the emetic pathway"
1993	First preclinical publications showing anti-emetic efficacy of a non-peptide NK_1 receptor antagonists (CP-99,994)	Ferret	Bountra et al., 1993; Tattersall et al., 1993	• These publications were from scientists at Glaxo and Merck but the compound used (CP-99, 994) was a Pfizer compound (Watson et al., 1995a,b). See text for details of other compounds and discussion of spectrum of anti-emetic effects.
1997	First clinical publication of anti-emetic effects on a non-peptide NK_1 receptor antagonist (CP-122,721) against high dose cisplatin chemotherapy	Human	Kris et al., 1997	• This study supported the translation of ferret data to human and demonstrated significant efficacy in the delayed phase, in contrast to the effects of 5-HT_3 receptor antagonists (see text for details)
2003	Approval of Aprepitant (Emend®) by European Medicines Evaluation Agency and Food and Drug Administration for treatment of emesis induced by cisplatin chemotherapy	Human		

For detailed discussion see Andrews and Rudd (2004).

pancreatitis (de la Puente-Redondo et al., 2007) as well as blocking vomiting induced by hydromorphone when used as a surgical premedication (Claude et al., 2014) and motion sickness (Benchaoui et al., 2007a). Maropitant is available for prevention of vomiting in cats (Batchelor et al., 2013). Other anti-emetics used in humans such as metoclopramide and ondansetron have also found veterinary use (Kenward et al., 2017).

A final note: Among all the proposed clinical indications for NK_1 receptor antagonists (especially pain, depression, anxiety, emesis), based on animal and human data (Kramer et al., 1998; Saria, 1999), only the anti-emetic indication successfully translated to clinical usage. For emesis at least, this activity was not subject to putative "promiscuity" among NK receptors for endogenous ligands (see above); NK_3 receptor antagonism did not inhibit cisplatin-evoked emesis in ferrets (King and Sanger, 2005).

NK_1 And 5-HT_3 Receptor Crosstalk

Palonosetron (RS 25259-197) was synthesized and characterized by Syntex Discovery Research (Clark et al., 1993; Eglen et al., 1995), before being licensed to Eisai and Helsinn for co-marketing in the USA in 2003 (the same year as aprepitant was approved by the EMEA and FDA). The drug has a relatively high binding affinity for the 5-HT_3 receptor (Wong et al., 1995; Muchatuta and Paech, 2009) and a long plasma half-life in healthy volunteers (Stoltz et al., 2004; Muchatuta and Paech, 2009). Surprisingly, palonosetron was effective in both acute and delayed phases of CINV. The drug did not antagonize the NK_1 receptor (Wong et al., 1995) and since other 5-HT_3 receptor antagonists did not have the same efficacy profile, research was initiated to explain these findings. This showed that in contrast to the first generation of 5-HT_3 receptor antagonists, which are competitive receptor antagonists, palonosetron binds allosterically to the receptor, exhibiting positive cooperativity; the authors argued that the difference in structure between palonosetron and the earlier 5-HT_3 receptor antagonists may, somehow, explain this difference (Rojas and Slusher, 2012). Further experiments demonstrated a persistent ability to inhibit receptor function after the drug was removed, triggering receptor internalization of the drug-receptor complex into the cell (Rojas et al., 2010). Since palonosetron remained bound to the 5-HT_3 receptor, this internalization now persisted for much longer than anticipated for a simple competitively-acting receptor ligand, raising the possibility that the internalized complex could interact and "crosstalk" with NK_1 receptor signaling pathways, inhibiting the functions of substance P (Rojas and Slusher, 2012; Rojas et al., 2014). Furthermore, palonosetron inhibited the upregulation of substance P expression in the nodose ganglia induced by cisplatin in rats, whereas granisetron and other 5-HT_3 receptor antagonists did not (Rojas and Slusher, 2012).

Interestingly, a possible interaction between 5-HT_3/NK_1 receptors had been demonstrated 10 years previously by Minami et al. (2001) using *in vivo* recording from ferret abdominal vagal afferents (e.g., Minami et al., 2001). This study showed that an NK_1 receptor antagonist (CP-99,994) reduced the afferent response to 5-HT and conversely the 5-HT_3 receptor antagonist granisetron reduced the afferent response to Substance P.

To date, palonosetron is the only 5-HT_3 receptor antagonist approved by the FDA for prevention of both acute and delayed CINV. The combination of palonosetron with NK_1 receptor antagonists such as netupitant therefore appears to have synergistic activity and good efficacy against both "acute" and "delayed" emesis (Rojas et al., 2014). Indeed, when these two drugs are given together with dexamethasone, total control of cisplatin-induced vomiting has been reported in the absence of significant nausea (Aapro et al., 2014; Keating, 2015). Today,

Helsinn markets an oral fixed-dose combination product of netupitant with palonosetron (NEPA) for prevention of CINV.

The experience with palonosteron demonstrates that the pharmacological profile of a compound defined at the time of discovery does not necessarily predict the *in vivo* effects.

CHALLENGES IN IDENTIFICATION OF NOVEL ANTI-EMETIC DRUGS

No Single Organ Target

Nausea and vomiting involve multiple organs and systems (e.g., visceral and somatic divisions of the peripheral nervous system, the digestive tract and respiratory system), including the central nervous system (CNS) which integrates the sensory inputs and motor outputs. Thus, there is no obvious single physiological pathway or organ to study, in contrast to asthma (airways), peptic ulcer (gastric and duodenal mucosa) and angina (coronary circulation). Pain, with sensory, behavioral, CNS, and motor components would be the most analogous clinical problem to nausea and vomiting.

The lack of a clear "target organ" means that it is difficult to apply modern molecular techniques for target identification and validation, and such methods have not (yet) contributed to anti-emetic drug discovery. Nevertheless, twin and (Reavley et al., 2006) genome-wide association studies (Hromatka et al., 2015) of motion sickness begin to illustrate the potential for molecular studies to provide insights into tractable targets.

Animal Models and Their Translational Value

The commonly-used laboratory rodent species do not vomit (Sanger et al., 2011; Horn et al., 2013) so most early research used non-human primates (particularly the squirrel monkey in motion sickness research) and dogs, with a few studies utilizing cats. Although dogs have been used for emesis research for at least 150 years (see Hatcher and Weiss, 1923 for review of early literature), in the last 35 years ferrets and to a lesser extent mink (both carnivores) have largely supplanted dogs for emesis research (see Percie du Sert and Andrews, 2014 for review of the history of their use in emesis research and references) although cats continue to be used for studies of motion sickness (e.g., Yates et al., 2014). The insectivore *Suncus murinus* (house musk shrew) has also been utilized, largely because it is highly sensitive to motion (Ueno et al., 1987, 1988) and its small size (<100 g) reduces the amount of a novel compound that needs to be synthesized for testing *in vivo*. Similarly, the least shrew (*Cryptotis parva*) which only weighs ∼5 g has also been utilized (e.g., Zhong et al., 2014). However, for most of these species their genome has not been sequenced, hampering translation of receptor pharmacology across species. It is also important to note that for an animal model to have translational value for humans, the species must respond to the same stimulus (preferably at doses comparable to those used clinically), must cause emesis by the same pathway/mechanism as in humans (bearing in mind that pathways may exhibit plasticity as the result of disease and the mechanism in humans may not be known) and must involve the same neurotransmitter and receptor sub-type in the pathway.

A critical question related to translation is *"Do Animals Experience Nausea and if so, How could it be Measured?"* The mechanical act of vomiting is broadly similar in humans and the laboratory animals. Until relatively recently, the ability of a substance to block retching and vomiting in an animal was taken as an indication that nausea was also likely to be blocked when tested in humans. For example, as some behaviors accompanying cytotoxic drug-induced emesis in ferrets were inhibited by 5-HT_3 receptor antagonists (e.g., burrowing and backing-up movements; Bermudez et al., 1988; Hawthorn and Cunningham, 1990; but see Lau et al., 2005a,b for more recent analysis) it seemed reasonable to suggest that 5-HT_3 receptor antagonists could also have anti-nausea effects in humans. However, it has since become apparent that 5-HT_3 receptor antagonists have a relatively lower efficacy against nausea induced by chemotherapy as opposed to vomiting (Soukop, 1990). Research in animals continues (there is considerable debate regarding nausea in animals and the nature of the assumed sensory experience) and many pre-clinical studies investigating mechanisms of emesis now include one or more of the measurements argued to be indices of nausea (e.g., Horn et al., 2011; Lu et al., 2017a,b; for detailed discussion of the issues see Stern et al., 2011, Chapter 8; Andrews and Sanger, 2014). Additionally, in animals, *post mortem* analysis of the pattern of activation of brain nuclei indicated by *c-Fos* immunohistochemistry can also give insights into which "higher" brain regions can be activated by an emetic, giving some insight into possible sensory experiences which may accompany vomiting and/or nausea (Lu et al., 2017b; Tu et al., 2017).

The Challenges of Research on Nausea and Vomiting in Humans

Studies of the physiology and pharmacology of nausea and vomiting in healthy volunteers are not common and usually involve use of relatively mild stimuli so only nausea is induced. These include apomorphine (Isaacs and MacArthur, 1954), ipecacuanha (Minton et al., 1993), and opioid receptor agonists (Soergel et al., 2014) together with motion, most commonly in the form of vection.

Although clinical trial design methodology is well-established, improved methodology for real-time, more objective and quantitative measurements of nausea and vomiting is needed in humans to improve characterization of the side effects of new treatments and better characterize the effects of anti-emetics, also facilitating more valid comparisons with pre-clinical studies.

Four areas appear promising for human research: (i) Improved understanding of the neuropharmacology of brain pathways implicated in nausea using brain imaging techniques combined with physiological studies of changes accompanying nausea (e.g., heart rate variability, plasma vasopressin and gastric motility); (ii) Analysis of large patient data sets to identify relationships between symptoms (e.g., nausea, early satiety) and underlying pathology (e.g., dysfunctional interstitial cells of Cajal within the stomach wall; see below); (iii) Precise characterization

of the efficacy of anti-emetics in specific patient sub-populations so that molecular correlates can be identified (e.g., 5-HT_{3B} receptor gene (Tremblay et al., 2003) and ABCB1 polymorphisms (Babaoglu et al., 2005; Tsuji et al., 2013) as predictors of 5-HT_3 receptor antagonist efficacy in CINV), potentially providing data to develop personalized therapies; (iv) Identification of the genomic/molecular basis for individual and population differences in sensitivity to different emetic stimuli; for example for motion sickness, which itself is a prognostic indicator for other causes of emesis (e.g., Warr, 2014), there are inter-individual (e.g., twin studies, Reavley et al., 2006), sex (female sensitivity > male, Lentz and Collins, 1977) and ethnic (greater sensitivity in Chinese subjects compared with African-American and Caucasian subjects, Stern et al., 1983) differences. Genome-wide association studies of large populations (>80,000 subjects) have begun to identify single nucleotide polymorphisms (SNP) associated with increased motion sickness sensitivity (Hromatka et al., 2015) and SNPs in the opioid receptor gene (*OPRM1*) have been associated with PONV (Sugino et al., 2014).

CURRENT AND FUTURE DIRECTIONS IN RESEARCH: LESSONS FROM THE PAST FOR THE FUTURE

Repurposing: Old Drugs for New Treatments

A number of the drugs described above were not "designed" as anti-emetics; this was discovered after their introduction for different therapeutic uses. More recently, there is now a growing list of other drugs which were originally used to treat psychosis and depression, and which have subsequently been shown to inhibit nausea and vomiting in several difficult-to-treat indications, including patients receiving palliative care. These include amitriptyline, levomepromazine, mirtazapine, olanzapine, and gabapentin. **Table 5** summarizes their discovery, original use approved by the FDA, the key pharmacology and their additional, "repurposed" use as anti-emetic drugs.

Nausea: Old and New Approaches (Table 3)

Nausea still remains relatively poorly treated in comparison to vomiting in many clinical settings including CINV (e.g., Jordan et al., 2016; Aapro, 2018) and there is an increasing recognition in the literature of its importance as a symptom (Donovan et al., 2016); a recent review on CINV posed the question "*Time for more emphasis on nausea?*" (Ng et al., 2014).

Gastric Emptying as a Target

Delayed gastric emptying can occur in diverse disorders (e.g., chronic renal failure and Parkinson's), but particularly those with a digestive tract etiology (e.g., gastroparesis, functional dyspepsia, scleroderma) in which nausea is also a symptom. Although evidence for a causal relationship between the genesis of nausea and delayed gastric emptying is inconsistent (Sanger and Pasricha, 2017) there has been a widely held (but also challenged, Sanger and Andrews, 2006; Sanger et al., 2013) assumption since the 1960s that restoring gastric emptying will alleviate the nausea (see McRitchie et al., 1984 for review); this forms the rationale for preferential use of prokinetic (and also anti-emetic) drugs such as metoclopramide (the longest approved drug for treatment of gastroparesis; Schulze-Delrieu, 1979; Camilleri et al., 2013) and domperidone (Brogden et al., 1982) to alleviate nausea (**Figure 7**). This approach has been pursued more recently by exploiting the gastric prokinetic properties of the antibiotic drugs erythromycin and azithromycin (Broad and Sanger, 2013), providing another example of "repurposing" and stimulating research into the prokinetic effects of macrolides (Broad et al., 2012). Nevertheless, until the precise mechanistic relationship between the various causes of delayed gastric emptying (e.g., disruption of the ENS, e.g., diabetic enteric neuropathy, Chandrasekharan and Srinivasan, 2007) and nausea is understood in a range of disorders, approaches based on prokinetics will continue to be more empirically, rather than rationally based.

Gastric Dysrhythmia as a Target

Movements of the human stomach muscles are regulated or "paced" by interstitial cells such as the interstitial cells of Cajal (ICC) which exist as different syncytia within the stomach wall (e.g., Rhee et al., 2011). In summary, these cells generate electrical slow waves which are transmitted to smooth muscle via gap junctions to create "waves" of muscle contraction that move from the gastric corpus down to the pyloric regions, promoting gastric emptying into the intestine (Blair et al., 2014). Increased understanding of their role in the etiology of gastric dysrhythmias linked to nausea (in which changes in functions lead to disrupted patterns of movements within different ICC/muscle syncytia without necessarily changing rates of gastric emptying; see Sanger and Pasricha 2017), particularly in conditions such as gastroparesis (Owyang and Hasler, 2002; Angeli et al., 2015), makes them an increasingly attractive target. The ion channels modulating functions of these cells (Lees-Green et al., 2011) are of particular interest as drug targets.

Appetite and Nausea Relationship

Most recently, research has focussed on the concept that nausea might be reduced by drugs which promote appetite, particularly as nausea, vomiting, pain, early satiety and bloating are a common symptom cluster in upper digestive tract disorders such as chronic dyspepsia and gastroparesis (Revicki et al., 2009). The hormone ghrelin has been shown to alleviate anorexia and vomiting in animal models and reduce cachexia and nausea in cancer patients, activities thought to be related to its ability to promote appetite (and perhaps "hedonistic eating" via a constitutively-active receptor; see Sanger and Furness, 2016). In two Phase II studies in patients with diabetic gastroparesis the ghrelin receptor agonist relamorelin, accelerated gastric emptying and reduced vomiting frequency and severity (Lembo et al., 2014, 2016).

Central Nervous System Pathways as a Target

The sensation of nausea requires activation of pathways in the cerebral hemispheres and most likely the cerebral cortex

TABLE 5 | Summary of key drugs "repurposed" for the control of emesis.

Discovery	Original use	Summary of pharmacology	Anti-emetic use
Amitriptyline			
• Discovered by several groups in 1960[1]	• Tricyclic antidepressant; approved by the FDA in 1961[2]	• Inhibits 5-HT and noradrenaline transporters • Also has affinity for the H_1 receptor, muscarinic receptors, the α_1-adrenoceptor and 5-HT_{2A} receptor, at concentrations similar to those which bind 5-HT and noradrenaline transporter sites[4]	• Reduced symptoms in patients with chronic nausea and vomiting (with pain as a predominant symptom) and in diabetic patients with unexplained vomiting[4,5]
Levomepromazine			
• Originally described by Rhone-Poulenc in 1956[6]	• Phenothiazine neuroleptic drug	• Can antagonize at H_1, muscarinic M_1/M_2, D_1, D_2, D_3 and D_4, receptors, the α_1 adrenoceptor and the $5HT_2$ receptor[7,8]	• Has found use in treatment of patients with intractable nausea and vomiting receiving palliative care where it is also used to treat severe delirium or agitation at the end of life[9]
Mirtazapine (Org 3770)			
• Synthesized in 2000[10]	• Antidepressant drug	• An antagonist at H_1, alpha$_2$ adrenoceptor, 5-HT_{2C}, 5-HT_{2A} and 5-HT_3 receptors[11] • Has affinity for several GPCRs, but has highest measurable affinity for α_2-adrenoceptors (IC_{50} order of potency: $_{2A} > _{2C} > _{2B}$) and 5-HT_{2A} and 5-HT_{2C} receptors (Ki order of potency 5-$HT_{2C} > _{2A} > _7 > _{1A}$ (**Table 2**).	• Several case reports and small studies suggest anti-emetic efficacy in patients undergoing surgery, suffering from hyperemesis gravidarum, chronic unexplained nausea and vomiting, and severe gastroparesis unresponsive to conventional treatments[12,13,14] • Also used to treat vomiting and co-morbid anxiety or depressive disorders in patients with chronic or cyclical vomiting syndromes[15]
Olanzapine			
• A thienobenzodiazepine originally described by Eli Lilly in 1980[16]	• Atypical antipsychotic	• Has affinity for M_1, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, M_4, $H_1 > M_3$, M_2, $D_2 > D_4$, D_1, α_1-adrenoceptor >5-HT_3[17,18,19]	• Used to prevent and treat breakthrough chemotherapy-induced emesis when given alone and in combination with other anti-emetic drugs[20] including patients receiving palliative care[21,22] • For example, a significant improvement in nausea reported when given together with 5-HT_3 and NK_1 receptor antagonists[23,24]
Gabapentin			
• Synthesized in 1974 (by Gerhard Satzinger) at Parke-Davis (now owned by Warner-Lambert/Pfizer) as potential epilepsy drug, incorporating γ-aminobutyric acid into a lipophilic cyclohexane ring to cross the blood-brain barrier	• Approved by the FDA in 1994 to control partial seizures and in 2002 for conditions with neuropathic pain[25,26]	• No mechanistic studies in emesis but its analgesic effects are attributed to blockade of the α_2/δ subunit of voltage-gated calcium channels[27]	• First reported as a potential drug to treat nausea in CINV in 2003 and subsequent studies have extended this to PONV and possibly hyperemesis gravidarum[27,28]

[1]*Sneader, 2005;* [2]*Fangmann et al., 2008;* [3]*Owens et al., 1997;* [4]*Prakash et al., 1998;* [5]*Sawhney et al., 2007;* [6]*Sigwald et al., 1956;* [7]*Lal et al., 1993;* [8]*Srivastava et al., 2009;* [9]*Dietz et al., 2013;* [10]*Kennis et al., 2000;* [11]*Anttila and Leinonen, 2001;* [12]*Hasler, 2016;* [13]*Kim et al., 2006;* [14]*Kundu et al., 2014;* [15]*Coskun and Alyanak, 2011;* [16]*Chakrabarti et al., 1980;* [17]*Bymaster et al., 1996;* [18]*Navari, 2014;* [19]*Leggio et al., 2016;* [20]*Chiu et al., 2016;* [21]*Atkinson, 2014;* [22]*MacIntosh, 2016;* [23]*Hocking and Kichenadasse, 2014;* [24]*Navari, 2014;* [25]*https://www.chemistryworld.com/podcasts/gabapentin/1017577.article;* [26]*Sirven, 2010;* [27]*Guttuso et al., 2003;* [28]*Guttuso, 2014.*

(Farmer et al., 2015). To block nausea initiated by activation of one of the classical input pathways (vestibular system, area postrema, vagal afferents) will require a drug which acts at some point along the pathway at which these outputs converge to project information to the cerebral hemispheres. The closer the drug acts to the cortical site of sensation genesis the greater will be the probability of treating nausea irrespective of the cause (including psychogenic). Although conceptually simple in approach we currently lack sufficiently detailed knowledge of the pathways in humans activated during nausea and their associated neurotransmitters and receptors.

CONCLUSION

The key steps in the identification and development of the current armamentarium of anti-emetic drugs reveal a number of recurrent themes with resonance in other drug discovery areas. These include: the use of traditional medicines as a basis

for new drugs; the frequent role of serendipity and exploitation of fortuitous observations; the impact of "non-research" issues such as mergers, takeovers, management decisions, patents, and associated litigation; the challenges of translation of animal models to the clinic in an area where there is no single target organ or molecular mechanism; the advances in pharmacology which change the binding profile and nature of receptor interactions of a drug (even after licensing); the repurposing of drugs active at multiple receptors for one indication but shown subsequently to exert an unanticipated profile of activity in another indication.

The last 30 years since the discovery of 5-HT_3 and NK_1 receptor antagonists has seen a major advance in the treatment of nausea and vomiting but major gaps remain including: (a) our understanding of nausea is poor in comparison to pain although it is arguably as common and debilitating, (b) the relationships between appetite, disordered gastric motility and nausea are still not understood, leading to a lack of advances in the treatment of common disorders such as gastroparesis and functional dyspepsia, and (c) there is no registered treatment specifically for nausea irrespective of cause or a "universal anti-emetic," a drug which would block both nausea and vomiting completely irrespective of the cause (Andrews and Sanger, 2014).

It is notable that the two major breakthroughs (involvement of 5-HT_3 and NK_1 receptors) in anti-emetics occurred within $<$10 years of each other and in the subsequent $>$20 years there has not been a comparable "major breakthrough;" why is this the case when molecular pharmacology has exploded over the same period? To some extent this can be explained by the success of the 5-HT_3 and NK_1 receptor antagonists in treatment of vomiting, but the same cannot be said for nausea, particularly in conditions such as gastroparesis. We might ask "where will serendipity now occur in an age when the mechanisms of action of drugs are much better understood?" Perhaps an answer can be found in studies looking for single-nucleotide polymorphisms associated with particular patient-symptom associations (e.g., sensitivity to motion sickness; see above).

A comment at a meeting to discuss anti-emetic agents for chemotherapy is pertinent to close: "I believe it is an interesting phenomenon that every 30 years everything done in the past is lost" (Dr. Lassner, p21S, in Penta et al., 1981); perhaps the answer to the question we posed about "where will new anti-emetics come from?" is already there in the recent history of this area.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Tamoxifen from Failed Contraceptive Pill to Best-Selling Breast Cancer Medicine: A Case-Study in Pharmaceutical Innovation

Viviane M. Quirke *

School of History, Philosophy and Culture, Faculty of Humanities and Social Sciences, Oxford Brookes University, Oxford, United Kingdom

Today, tamoxifen is one of the world's best-selling hormonal breast cancer drugs. However, it was not always so. Compound ICI 46,474 (as it was first known) was synthesized in 1962 within a project to develop a contraceptive pill in the pharmaceutical laboratories of ICI (now part of AstraZeneca). Although designed to act as an anti-estrogen, the compound stimulated, rather than suppressed ovulation in women. This, and the fact that it could not be patented in the USA, its largest potential market, meant that ICI nearly stopped the project. It was saved partly because the team's leader, Arthur Walpole, threatened to resign, and pressed on with another project: to develop tamoxifen as a treatment for breast cancer. Even then, its market appeared small, because at first it was mainly used as a palliative treatment for advanced breast cancer. An important turning point in tamoxifen's journey from orphan drug to best-selling medicine occurred in the 1980s, when clinical trials showed that it was also useful as an adjuvant to surgery and chemotherapy in the early stages of the disease. Later, trials demonstrated that it could prevent its occurrence or re-occurrence in women at high risk of breast cancer. Thus, it became the first preventive for any cancer, helping to establish the broader principles of chemoprevention, and extending the market for tamoxifen and similar drugs further still. Using tamoxifen as a case study, this paper discusses the limits of the rational approach to drug design, the role of human actors, and the series of feedback loops between bench and bedside that underpins pharmaceutical innovation. The paper also highlights the complex evaluation and management of risk that are involved in all therapies, but more especially perhaps in life-threatening and emotion-laden diseases like cancer.

Keywords: synthetic anti-estrogen, contraceptive pill, breast cancer, chemoprevention, risk evaluation and management, adjuvant therapy

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***Correspondence:**
Viviane M. Quirke
vquirke@brookes.ac.uk

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INTRODUCTION

Today, tamoxifen (brand name Nolvadex) is one of the world's best-selling hormonal breast cancer drugs. However, it was not always so. Compound ICI 46,474 (as it was first known) was synthesized in 1962, quite unusually for the time, by a female chemist: Dora Richardson, who was responsible for making triphenylethylene derivatives within a project to develop a contraceptive pill in the pharmaceutical laboratories of the British chemical group ICI (now part of AstraZeneca). Although designed to act as an anti-estrogen, the compound was found to stimulate, rather than suppress

ovulation in women. This, and the fact that at first it could not be patented in the USA, its largest potential market, meant that ICI nearly stopped the project. If it was saved, it was partly because the team's leader, Arthur Walpole, threatened to resign, and pressed on with another project: to develop tamoxifen as a treatment for breast cancer. Even then, its market appeared small, because at first it was mainly used as a palliative treatment for advanced breast cancer. An important turning point in tamoxifen's journey from orphan drug to best-selling medicine occurred in the 1980s, when the results of clinical trials showed that it was also useful as an adjuvant to other forms of therapy in the early stages of the disease. Later, trials demonstrated that it could prevent its occurrence or re-occurrence in women at high risk of developing breast cancer. Thus, it became the first chemopreventative for any cancer, helping to establish the broader principles of chemoprevention, and extending the market for tamoxifen and similar drugs further still.

Hailed as a pioneering medicine that has saved the lives of thousands of women[1], much has been written about tamoxifen, especially in recent years by Craig Jordan, the researcher who was influential in the latter part of its history (Maximov et al., 2016). However, as the public's and the medical profession's dependence on drugs not only to treat, but also to prevent an ever growing variety of conditions has come under increasing scrutiny (Greene, 2007), tamoxifen has also been investigated by sociologists as an example of what they describe as the "biomedicalization" of society, i.e., the shift in the use and meaning of drugs from treatment to prevention, involving a cost-benefit calculation that is seldom openly discussed (Fosket, 2010, pp. 341–348; Löwy, 2010, pp. 185–188; Löwy, 2012).

There is another strand in the literature, which is somewhat less well-developed, and concerns tamoxifen at once as an emblematic and an idiosyncratic example of pharmaceutical innovation. For the history of tamoxifen suggests a model of pharmaceutical innovation that is far more complex than a linear model from bench to bedside (Schwartzman, 1976; Howells and Neary, 1988; Gambardella, 1995; Landau et al., 1999). Rather, it incorporates numerous dead ends, feedback loops, as well as serendipitous observations made by individual researchers (and associated with other discoveries, in this instance the isolation of the estrogen receptor). Hence, the scientists whose work has shaped pharmaceutical innovation are an important part of the story—in the case of tamoxifen, not only Richardson, but also Walpole, the biologist who led the research team at ICI and provided the link between the different projects within which tamoxifen was developed (Jordan, 1988), and, for the later stage in the drug's tortuous journey, Jordan.

At a time when the drying up of old drug pipelines has led to anxieties about the end of the Therapeutic Revolution and the need to find new models of drug discovery to replace those which produced many of the blockbuster drugs we know today, tamoxifen therefore presents an opportunity to explore the historically contingent nature of pharmaceutical innovation, addressing several of the questions posed by the editors (see their introduction to this special issue). Using the research and development reports of the company that developed the drug (ICI)[2], an unpublished history of tamoxifen, written by Richardson and accompanied by letters from patients[3], as well as some of the numerous publications on the topic, the paper will show how the early history of the drug shaped its fate in the medical marketplace, and therefore deserves to be better understood than it is at present. The paper argues that its origins as a contraceptive pill rather than a cancer remedy meant that concerns over side-effects, alongside its ability to counteract the action of estrogen, dominated the company's research and development agenda. Hence patients' voices, which provided indications the drug's safety and efficacy at once directly and indirectly, helped to define this agenda, and the absence of side-effects relative to its anti-estrogenic activity would become one of the key selling points of tamoxifen as an anti-cancer drug compared to alternative treatments.

However, because of its very ability to prolong life in women suffering from breast cancer, tamoxifen was later found to have a number of potentially serious long-term side-effects, which range from pulmonary thrombosis to endometrial cancer. Nevertheless, its usefulness in treating and preventing a major cause of death in women has meant that, to this day, it remains on the WHO's List of Essential Medicines (WHO, 2015). This paper will therefore also highlight the complex evaluation of risk that is involved in all therapies, but more especially perhaps in diseases as threatening and emotionally charged as cancer, not only at the regulatory and clinical levels, but also at the individual level of the patient.

RESULTS

Before focusing on the development of tamoxifen, it is useful to describe the background for the different projects that led first to its synthesis, second to its early trajectory as an anticancer drug, for it illustrates not only the non-linear nature of pharmaceutical innovation, but also the lengthy accumulation of in-house scientific knowledge and technical know-how which underpins it, and yet is rarely brought to the fore in histories of drug discovery (Weatherall, 1990; Sneader, 2005; Ravina, 2011).

The Use of Sex Hormones and Synthetic Analogues in Cancer

The link between hormones and cancer has been known at least since 1916 (Lathrob and Loeb, 1916). However, their usage in the treatment of cancer depended on their isolation, purification and chemical determination, which was not achieved until the 1930s in the case of sex hormones. One such hormone was the follicular hormone (Follicle-Stimulating Hormone, FSH), which was prepared by the Roussel Laboratories, a French company

[1]In 2003 it was estimated that 400,000 women were alive thanks to the drug, and millions have benefited from extended disease-free survival (See Jordan, 2003).

[2]AstraZeneca, formerly ICI (hereafter AZ), research and other reports: Oral Contraception (AZ CPR 70: 1960-64); Endocrinology and Fertility (AZ CPR 101: 1965-72); Viruses and Cancer (AZ CPR 54-55: 1958-64). These unpublished reports were made accessible to me between 2002 and 2009 by kind permission from AstraZeneca.

[3]D. N. Richardson, "The history of Nolvadex" (AZ PH27039 B, 13 May 1980).

specializing in biologicals, and supplied to Antoine Lacassagne at the Institut du Radium in Paris. Using this hormone, Lacassagne was able to show a direct link between estrogens and the appearance of breast cancer in mice (Lacassagne, 1932, 1936). But natural estrogens were difficult to obtain in the quantities required for large-scale experiments. A major turning point occurred when E.C. (later Sir Charles) Dodds, working at the Middlesex Hospital in London in collaboration with researchers at the Dyson Perrins Laboratory in Oxford, discovered that the synthetic compound stilboestrol had estrogenic properties (Dodds et al., 1938). Dorothy Crowfoot (later known by her married name, Hodgkin), who worked nearby at Oxford University's Inorganic Chemistry Department, established using X-ray crysallography that its chemical structure resembled estrogen (Carlisle and Crowfoot, 1941). Inexpensive to make and apparently well tolerated in patients, stilboestrol was therefore widely prescribed for cases of estrogen deficiency, especially in menopausal women (Sneader, 2005, p. 197). Although it would later be linked to cases of vaginal or cervical adenosarcomas in daughters of women who had been prescribed the drug in their first trimester of pregnancy (to avoid unwanted abortion; see Gaudillière, 2014), it was also the first synthetic drug to be used for treating cancer (Weatherall, 1990, pp. 217–218). Indeed, in 1939, Charles Huggins of the University of Chicago successfully treated cases of prostate cancer with stilboestrol (known as diethylstilbestrol in the US; Huggins and Hodges, 1941), and by 1950 a co-operative trial had shown that the synthetic estrogen was effective in delaying the progress of this type of malignant disease (Nesbitt and Baum, 1950). However, breast cancer proved more difficult to treat, as it could either be inhibited or stimulated by administration of estrogen.

Following the publication of Dodds' findings, synthetic substances with a similar structure were examined for estrogenic activity, such as, triphenylethylene. These substances, which could not only be mass produced, but also be chemically modified to obtain derivatives with *anti*-estrogenic activity, therefore became compounds of choice for studies in Britain and elsewhere. One of the organizations that studied it was ICI, which I turn to now.

ICI and Cancer Research

The company's interest in cancer was a long-standing one. When triphenylethylene was found by Charles Scott, a researcher in Edinburgh, not only to be active by mouth, like stilboestrol, but to have a durable estrogenic action, and therefore to have potential as an alternative to stilboestrol, Arthur Walpole, a biologist who had joined ICI's Medicinal Section in 1938, began carrying out some exploratory work with the substance. This work led to the synthesis of various triphenylethylene derivatives, including triphenylmethylethylene (M 612) and triphenylchloroethylene (registered in 1940 under the name Gynosone)[4]. In 1942, these compounds were supplied by the company for trials in breast cancer to Alexander Haddow of the Chester Beatty Institute in London, Edith Patterson at the Christie Hospital in Manchester and their collaborators. Although improvements were only temporary, there was clear evidence that Gynosone in particular caused regression and therefore could be beneficial in the treatment of breast cancer (Haddow et al., 1944; Walpole and Paterson, 1949).

Meanwhile, on the other side of the Atlantic, the compounds known as "nitrogen mustards," which were being studied as part of a chemical warfare research programme, were shown to inhibit the growth of blood and lymph tumors by Goodman and Gilman at the University of Yale, a discovery often hailed as the beginning of cancer chemotherapy[5]. Despite this wartime work being top-secret, Walpole and Haddow were also able to investigate these compounds, thanks to an Anglo-American agreement to exchange scientific information (Weatherall, 1990, p. 218). Another, parallel study relating to cancer at ICI involved anti-metabolites. Following the discovery that ICI's novel anti-malarial drug Paludrine was converted in the body to cycloguanine, an active metabolite which interferes with purine biosynthesis, and spurred by the announcement that Burroughs Wellcome's drug 6-MP was effective against leukemia, the search for anti-metabolites began at ICI under the leadership of Frank Rose, who had run their anti-malarial programme during the war. Rose became Research Manager of the Chemistry Department in 1954, whilst remaining involved in bench work. As well as the search for alkylating agents, synthetic estrogens, and anti-metabolites, Rose also encouraged investigations into carcinogenesis, which was a rare interest for researchers working on cancer chemotherapy at that time (Suckling and Langley, 1990, pp. 507–508).

At first, ICI's approach to cancer was therefore largely empirical, involving the synthesis of derivatives of compounds that had known anti-tumor properties, without a formal cancer research programme. However, once plans had been made to build a pharmaceutical research center at Alderley Park near Manchester, and ICI started organizing its research in team projects, Cancer became such a project in 1955. The project was entitled "Cancer and Viruses: antibacterials[6]," and its team leader was the biologist E. Weston Hurst. Alderley Park opened in 1957, and between 1957 and 1960 Cancer and Viruses separated into two different projects.

During that time Cancer was merged with a new project to find an oral contraceptive, led by Arthur Walpole. Then, in 1960, the discovery of the natural antiviral substance interferon, and ICI's involvement in its study in collaboration with the Medical Research Council (see Pieters, 2005, Chapters 5–6), led to Viruses and Cancer coming together again. Oral Contraception therefore split away from Cancer, with Walpole working in parallel on both projects. His involvement in the Oral Contraception project (which in 1963 was re-named "Endocrinology," and later "Fertility," reflecting a gradual change in the research emphasis) would ensure that breast cancer remained an important focus for both his teams. It was within

[4]Richardson, "The history of Nolvadex."

[5]There compounds were later understood to work by alkylation—i.e., the transfer of an alkyl group from one molecule to another, in the case of anti-cancer agents attaching it to DNA, thus inhibiting cancer cell division—hence such compounds became known as "alkylating agents" because of their mechanism of action.

[6]Since the 1930s it was known that viruses can induce tumors in laboratory mice. The oncogenic potential of a number of virus groups, including adenoviruses, herpesviruses, and poxviruses, was identified in the 1950s and 1960s (See Rigby and Wilkie, 1985).

this Oral Contraception project that tamoxifen (Nolvadex), a triphenylethylene derivative, was synthesized and subsequently developed, initially as a contraceptive pill.

ICI, Oral Contraception, and the Origins of Tamoxifen

The first contraceptive pill had been synthesized in the early 1950s, and in 1956 Walpole wrote a survey entitled "The technical possibility of oral contraception[7]," which—as had become customary within ICI by that time (see Quirke, 2005)—gave an overview of the field to enable ICI to decide whether or not it was worth entering.

Walpole began by introducing the context in which such a pill would be developed. In doing so, he showed the extent to which contemporary concerns, which included anxiety over population growth, a decrease in death rates, food shortages, and an awareness of important differences between the developed and developing world, were internalized and acted upon by companies such as ICI. Then, in the main body of his report, Walpole enumerated the requirements for contraception:

1. it should not "offend social or religious scruples" and as little as possible the "aesthetic feelings" of those who might wish to avail themselves of it (however, he added, such considerations remained outside the scope of experimental biology);
2. it should be cheap enough to be readily available and simple enough to use by any people "intelligent enough to realize the possible consequence of coitus and to know whether or not they wished to conceive";
3. it should be effective over a known period of time with no prejudice to subsequent fertility;
4. it should not depend on a local action contemporaneous with coitus or any form of treatment which must be timed in a complex or critical manner in relation to the menstrual cycle;
5. it should involve only occasional dosage by mouth.

After describing what at the time was understood about the physiology of reproduction, he went on to list the technical possibilities of contraception at different stages in the reproductive cycle, from (a) spermatogenesis and sperm, to (b) ovulation and ovum, (c) fertilization, (d) fertilized ovum, (e) implantation of embryo, and lastly (f) development of embryo.

On the basis of substances already known to act as contraceptives, he concluded that it "would seem possible to produce temporary infertility in men by giving androgens, and of these methylsterone is active by mouth." He added that it was also possible "either to prevent conception or interrupt pregnancy at a very early stage in women by giving estrogens by mouth," but that such treatments must be free from undesirable side-effects. Among the newer partly synthesized steroids now becoming available, he believed that substances might be found that were be more specifically antagonistic toward progesterone (anti-progestins), and he argued that these would seem more suitable for continued use[8]. Other substances from natural sources, such as, the *Lithospermum ruderale,* a North American plant with a small white flower that could also be found in English hedgerows and was being investigated at the time by the Medical Research Council (Marks, 2001, pp. 49–50), appeared to him as "rather more suspect," and he acknowledged that clinical evidence was lacking, not only concerning these natural compounds, but also human contraception more generally.

As to the other substances that might be considered for contraception, toxicity was a major problem, such as the anti-folic drug aminopterin, for not only did it act as an early abortifacient, but it carried serious toxic hazards, like some of the other anti-metabolites. Similar concerns were associated with biological alkylating agents, which were potentially mutagenic and carcinogenic. Hence, taking into account both the requirements for contraception and the need to avoid toxic effects, especially since contraceptive substances were intended for use in normally young and healthy adults (Oudshoorn, 2002, pp. 123–157), the search for triphenylethylene derivatives, alongside investigations of natural and part-synthesized steroids, became the preferred course of action, as evidenced by ICI's research reports[9].

ICI were not alone in pursuing the triphenylethylene route. Indeed, when Leonard Lerner, a researcher working on a cardiovascular research program at the American drug company Merrell, reported in 1958 that a newly synthesized compound, MER 25 (ethamoxytriphetol), not only resembled structurally triphenylethylene, but had anti-estrogenic activity on both spayed and intact female rats, his discovery stimulated laboratory research and clinical investigation of other potential anti-fertility agents among triphenylethylene derivatives. ICI considered acquiring the drug under license from Merrell in order to study and potentially exploit it as a contraceptive, but interest in it waned, for in the meantime ICI had found that another compound, ICI 22,365 [N:N-bis (allylthiocarbamyl) hydrazine], which they employed in analytical chemistry and were currently investigating as an anti-parasitic for use in the poultry industry, prevented the development of sex organs and secondary characteristics such as the emergence of combs in chicks[10]. This finding led Walpole's team, which at that stage included G. E. Paget and J. K. Walley working on the biological side (while Dora Richardson and G. A. Snow worked on the chemistry), to test the compound in male and female rats, producing evidence that it caused a selective and reversible inhibition of the gonadotrophic functions of the pituitary in rats, and prevented pregnancy either by inhibiting ovulation, or by preventing implantation (the precise mechanism of action was yet unclear). In a report written in September 1960, Walpole wrote that the compound not only provided an interesting lead in oral contraception, but also in hormone-dependent cancers of

[7]Walpole, "The technical possibility of oral contraception" (AZ ICP 10695, 13 June 1956).

[8]G.D. Searle's norethynodrel, brand name Enovid, had been patented in 1955, and Syntex's norethisterone, brand name Ortho-Novum, was patented in 1956. NB: both were progesterone analogues containing some oestrogen (See Marks, 2001, pp. 72–73).

[9]Walpole, "Steroids as oral contraceptives" (PD/B 353, 13 Nov. 1958); AZ CPR 70 "Oral Contraception."

[10]Richardson, "The history of Nolvadex"—MER 25 was also later shown in clinical trials to have low potency and unacceptable side effects.

the prostate and breast, and it was decided that "if an alternative patentable compound were found which, in laboratory tests, proved superior (or even equivalent to it), then this compound should replace 22,365 in clinical studies[11]."

The most promising compound to come out of this programme, ICI 33,828 (which had a similar structure to 22,365), was therefore tested in pre-menopausal patients with mammary carcinoma, which was justified on the grounds that it might have a therapeutic as well as an anti-fertility effect. It was also tried in prostatic cancer, however the clinicians involved in these trials at the MRC Clinical Endocrinology Unit in Edinburgh received complaints from patients about nausea, anorexia, and occasional vomiting. Walpole also discovered that, before trials with 33,828 could begin, 22,365 had been given in November 1960 to a psychotic patient who was 15 weeks pregnant in order to induce abortion. However, the drug had failed to terminate the pregnancy, and estrogen excretion had remained unaffected by the treatment. The fetus, which had therefore had to be removed surgically, appeared normal. At the same time as plans for more extensive clinical studies, preferably closer to home so that his team could be more directly involved in the trials, Walpole therefore also made plans to develop more sensitive assay methods for gonatrophins in urine, blood, and pituitary, to better assess the clinical effects of their lead compound, and obtain more reliable measures of activity in animal experiments[12]. Shortly afterwards, in 1962, Mike Harper, a young endocrinologist who would play a significant part in the tamoxifen story, was invited to join the team.

Meanwhile, at Merrell, researchers had pressed on with the search for novel triphenylethylenes and in 1961 discovered that MRL 41 (also known as clomiphene, or chloramiphene, brand name Clomid), which was in fact an ether derivative of Gynosone, also inhibited pituitary gonadotrophins although it showed weak estrogenic activity. Remembering the earlier trials with Gynosone and M 612, Walpole therefore suggested to his team that they develop and examine an ether derivative of M 612[13]. The compounds they prepared in 1961 not only inhibited implantation of the fertilized ovum in the rat at a low dose (below that at which they would show estrogenic activity), but with the addition of a methoxy group they also had a greater duration of action. After the arrival of Harper, whose new series of biological tests helped to produce a clearer picture of the structure–activity relationships of triphenylethylenes, the programme of chemical synthesis was therefore stepped up. The team had grown, and as well as Walpole, Walley, and Richardson, it now included several members of the Biology Group: A. M. Barrett, M. J. K. Harper, G. E. Paget, Miss J. M. Peters, and J. M. Thorp, of the Chemistry Group: R. Clarkson, E. R. H. Jones, J. K. Landquist, B. W. Langley, W. S. Waring, and of the Biochemistry Group: W. A. M. Duncan. It was hoped that, with such increased resources, ICI could improve upon both clomiphene and a new Upjohn product with similar activity, U 11,555, by finding alternatives with less estrogenic and pituitary-inhibitory activity relative to their anti-fertility activity. For, by then, clinical studies of ICI 33,828 had produced disappointing results: not only did it have unpleasant and worrying side effects (nausea, drowsiness, a fall in thyroid function measured by thyroidal I^{132} uptake, and a rise in serum cholesterol)[14], but the inhibition of ovulation could not be achieved without suppressing menstruation, which made it undesirable as an oral contraceptive in women[15].

Among the newly synthesized triphenylethylenes, Harper drew up a short list for further study, primarily as potential anti-fertility agents. These included the dimethylamino ethoxy compound ICI 46,474 (later known as tamoxifen, brand name Nolvadex). It had been synthesized in 1962 by Richardson, and Harper selected it for additional tests and for preliminary toxicity studies. At the same time, the company lodged patent applications to protect ICI 46,474 and related compounds from competitors[16]. As well as providing basic data on these compounds, Patent GB1013907 covered a number of potential therapeutic uses, including cancer. It read:

> The alkene derivatives of the invention are useful for the modification of the endocrine status in man and animals and they may be useful for the control of hormone-dependent tumors or for the management of the sexual cycle and aberrations thereof. They will also have useful hypocholesteraemic activity[17].

ICI 46,474 (1962–67)

Although marred by a number of dead ends, which were partly due to ICI's strategy of closely following their competitor's activities and using their compounds as leads in the search for new, patentable products, the early phase of the Oral Contraception programme shaped tamoxifen and determined its future in many ways. The compounds developed within this programme were designed to act as contraceptive pills, yet from the beginning their usefulness in breast cancer was explored in close parallel. This dual objective was pursued as a result of Walpole's own research interests, and thanks to the fruitful collaborations he established both with endocrinologists and with clinicians working in cancer. The feedback loops between bench and bedside which this relationship created, and which led to a series of twists and turns that would become the hallmark of the tamoxifen story, meant that the compounds functioned both as research tools to study hormone function and metabolism in the laboratory, and as experimental treatments in the clinic. Importantly, the dual objective of developing a contraceptive pill whilst assessing the usefulness of compounds in breast cancer (even if as we have seen this was also a means of testing drugs

[11] A. L. Walpole and G.E. Paget, "Pituitary inhibitors" (AZ CPR 70/1B: Oral Contraception Sep. 1960).

[12] Walpole et al. "Pituitary Inhibitors" (AZ CPR 70/2B: Endocrinology, June 1961). NB: the reports were re-named "endocrinology" after the study of steroids and their action on cholesterol metabolism was included in the project.

[13] Richardson, "The history of Nolvadex."

[14] Cholesterol levels had become a serious concern since Merrell's new drug MER 29 (Triparanol) had been found to cause irreversible cataracts by interfering with cholesterol biosynthesis, and had had to be withdrawn from the market. B. W. Langley and A. L. Walpole (AZ CPR 70/5B: Endocrinology May 1963). See also Richardson, "The history of Nolvadex."

[15] B. W. Langley and A. L. Walpole (AZ CPR 70/5B: Endocrinology May 1963).

[16] The compounds were covered by patents GB 1013907 and 1064629.

[17] Quoted in Jordan (2006), p. 39.

before administering them to healthy women), also meant a constant preoccupation with side effects, and the low toxicity of tamoxifen relative to its potency would turn out to be one of its crucial advantages over its competitors.

A triphenylethylene derivative, with groups and side chains to enhance its anti-estrogenic and pituitary-inhibitory effect and prolong its duration of action, without interfering with its anti-fertility activity, ICI 46,474 had been demonstrated as the most potent and least toxic of all the compounds tested by June 1964[18]. But what exactly was it? In the process of gathering data for patent applications, scaling up production and preparing a submission to the newly formed Committee on Safety of Medicines (CSD), uncertainty arose as to the precise structure of the compound. Using an NMR spectrometer recently acquired by the company, in 1964 G. R. Bedford, a spectroscopist who had joined ICI's Pharmaceutical Division in 1963, showed that many of the active compounds synthesized so far were a mixture of isomers. However, it was unclear in which isomer the anti-estrogenic activity resided (did it reside in the *cis*, or the *trans* isomer?). The isomers were separated by fractional crystallization by Richardson. This represented quite a feat at the time[19], and revealed ICI 46,474 to be more active as an anti-implantation agent than its *cis* isomer ICI 47,699, which was more estrogenic (Bedford and Richardson, 1966; Harper and Walpole, 1966). In the meantime, Merrell had carried out a spectroscopic analysis of their own drug clomiphene, and disagreed with ICI's interpretation of the spectroscopic data, attributing the anti-estrogenic activity to the *cis,* not the *trans* isomer. The controversy led to some confusion among researchers, and eventually the matter was settled by X-ray analysis, which confirmed ICI's findings that the anti-estrogenic activity did indeed reside in ICI 46,474, that is to say in the *trans* isomer of the compound (Kilbourn et al., 1968).

So how did tamoxifen work? Before making a submission to the CSD, which in the wake of the thalidomide disaster had been set up to review all laboratory data on potential drugs in advance of their introduction into human patients, a basic understanding of their mechanism of action, as well as knowledge about any toxic effects, had to be achieved (see Quirke, 2012a). Therefore, unsurprisingly perhaps since it was intended for use in contraception, the first teratogenic test ever to be performed by ICI was carried out with tamoxifen. At the very low doses necessary to allow implantation of the fertilized ovum, rat offspring developed a deformity called "kinky ribs." However no such effects could be seen in rabbits or in primates, and it was later concluded that since ICI 46,474 restricts uterine growth, the deformity was caused by mechanical contraction and therefore could not considered a true teratogenic effect[20].

Tamoxifen was most effective in preventing implantation in rats when given on day 4 of the pregnancy, and virtually inactive on day 5. This suggested that it acted by interfering with a crucial event that had already occurred by the 5th day. It was suspected that ICI 46,474 prevented implantation by interfering with the critical estrogen release on the uterus that occurs between 12 and 20–21 h on the 4th day[21]. However, it was unclear whether the estrogen released at this time acted directly on the uterus or whether its action was mediated by vasodilating amines such as histamine. As there was evidence to support the latter hypothesis, ICI 46,474 was thought to act either as a direct estrogen antagonist, or by preventing the release of histamine, or as an antagonist of the amine. To explore this hypothesis, whilst carrying out further toxicity tests, experiments were devised in additional animal species (as well as rats, in mice, rabbits, dogs, monkeys, and sheep, for by then the compound was also being considered for use in veterinary medicine)[22]. These experiments revealed considerable species specificity, and by 1965 doubts had arisen whether an "estrogen surge" was necessary for ovo-implantation in humans, as it was in rats, and whether at the dosage required to oppose estrogen sufficiently to inhibit implantation ICI 46,474 would cause menstrual irregularities, therefore whether the compound would prove effective and be acceptable as an oral contraceptive[23]. Although it was still hoped that ICI 46.474 would provide a welcome alternative to the now familiar method of using mixtures of orally active estrogens and gestagens (also known as progestogens) to inhibit ovulation while at the same time producing withdrawal bleeding to replace spontaneous menstruation, a method which was considered too costly and too complicated for use in underdeveloped communities, it was felt that such doubts could only be "settled in the clinic[24]." However, first, the team needed to ascertain whether or not ICI 46,474 would produce irreversible damage to the ovaries or uterus, and for this studies in monkeys, particularly pig-tail monkeys in which changes in the reproductive cycle were found to most closely resemble those in man[25], were deemed to be the most helpful.

The First Collaborative Trials (1967–71)

While these further studies were being carried out, ICI began planning a trial with Dr. Klopper at Aberdeen, for the induction of ovulation in amenorrheic women rather than contraception[26]. Indeed, by then, clomiphene had been found to stimulate ovulation and prolong luteal function in amenorrheic women, and in 1967 was approved for the treatment of infertility in the US[27]. Moreover, obtaining approval to evaluate ICI 46,474 in oral contraception was problematic, not only because it involved long-term administration, but because of persisting fears among British gynecologists that it might lead to fetal malformation. In their eyes, unlike the conventional pill which contained

[18] Walpole et al. "Reproduction" (AZ CPR 70/6B: Endocrinology, June 1964).

[19] John Patterson, personal communication, 20 April 2009.

[20] Richardson, "The history of Nolvadex." It was later discovered that this effect disappeared by the time of weaning (Jordan, 2006).

[21] Walpole et al. "Reproduction" (AZ CPR 70/6B: Endocrinology June 1964).

[22] After it had been found to be estrogenic in sheep, in 1970 the compound was even considered as a fattening agent for livestock. Walpole et al. (AZ CPR 101/16B Endocrinology and Fertility 20 Feb. 1970).

[23] AZ CPR 101/1B Endocrinology and Fertility January 1965.

[24] Ibid.

[25] Walpole et al. (AZ CPR 101/20b Endocrinology and Fertility 28 June 1971). These changes were measured by radio-immunoassay (plasma estradiol) as well as protein binding (progesterone) once appropriate tests had been developed.

[26] AZ CPR 101/7B Endocrinology and Fertility, January 1967.

[27] Its introduction is said to have begun the era of assisted reproductive technology. See Dickley and Holtkamp (1996).

familiar ingredients such as estrogens and progestins that had traditionally been given to pregnant women without harm to the fetus, evidence of a lack of teratogenic effect in animal experiments with an unknown compound like ICI 46,474 did not constitute an adequate safeguard. Therefore, they believed that the first women to receive ICI 46,474 as a contraceptive must be offered an abortion, but under the terms of the 1967 Abortion Act this could only be offered to a very limited number of women[28]. Two solutions to this conundrum were envisaged: (1) to arrange a consortium of gynecologists to contribute such patients to a central unit in the hope of collecting a reasonable number fairly quickly; (2) to go abroad to a country, such as Hungary, where abortion was accepted as a means of population control. Meanwhile, therapeutic studies would be conducted to provide the sort of doses to be used in contraceptive trials, and approval to carry these out was obtained from the CSM in 1969. These studies included ICI 46,474 (now also referred to by its brand name Nolvadex) for the treatment of anovulation or menorrhagia associated with high levels of endogenous estrogen (to be carried out at Aberdeen, Manchester and the Women's Hospital in Chelsea), and of breast carcinoma in 30 menopausal and post-menopausal women (at the Christie Hospital in Manchester).

The preliminary reports received from Dr. Klopper in Aberdeen and Drs. Murray and Osmond-Clarke in London helped to cast further light on the drug's mechanism of action, showing that that tamoxifen was capable of inducing ovulation at higher dose levels, while at lower doses it tended to have an anti-estrogenic effect[29]. As to the Christie breast cancer trial, although two of the women complained about hot flushes (which was taken as evidence of its anti-estrogen effect), no toxicity was observed and the drug appeared to be well tolerated, even at the highest dose of 10 mg by mouth.

In her unpublished history of tamoxifen, Dora Richardson wrote of the team's excitement as the first trial results arrived. She described the news of the birth of a child to a woman who had been infertile for 12 years and had failed to respond to treatment with clomiphene as a "boost to morale[30]." She also described how the team were encouraged by the results of the breast cancer trial, even though these results were not received with universal enthusiasm at ICI: Walpole and his colleagues were told that they were supposed to be looking for a contraceptive pill, not an anti-cancer agent! At a Development meeting on 28th August 1970, sales estimates and quantities of bulk drug were set at 2 kg for initial stocks. Richardson concluded from these figures that the Development Department obviously envisaged treating only "dead people," an indication of the hopelessness of the condition as it was viewed at that time (as well as lack of faith or ignorance on the part of the Development team)[31]. However, fortunately, on the basis of the positive clinical results, the CSM granted the company permission to prolong the trials as well as extend them to other centers. By the end of 1970, 60 patients had been admitted to the Christie breast cancer trial, and of the 40 women who had been on the trial for more than 10 weeks, all had shown measurable and marked tumor regression. Although these results were comparable to those achieved with the established synthetic hormone diethylstilboestrol, the clinicians carrying out the trial, Drs. Todd and Cole, reported how impressed they were with the absence of toxicity and the low incidence as well as trivial nature of any side-effects (Cole et al., 1971), especially compared with other agents used in cancer at the time, which were often either toxic, or—in the case of breast cancer—tended to have androgenic effects, and in some instances were so intolerable that patients had been withdrawn from treatment[32].

In return, the trials provided clinical material for laboratory studies of tamoxifen. By then, the estrogen receptor had been isolated and identified by Gorski (Gorski et al., 1968), and Walpole and his team developed a receptor protein-binding assay method[33]. However, in a clinical setting, it was felt that a radio-immunoassay was more specific for measuring blood-estradiol levels in patients given tamoxifen[34]. The receptor-protein binding assay was therefore mainly used for experiments in laboratory animals, and showed tamoxifen to be a competitive inhibitor of estradiol binding to the uterine receptor protein in rabbits and in mice. Receptors sensitive to anti-estrogen were also found in various parts of rats' brains, including the hypothalamus and the pituitary. The results of the receptor-protein binding experiments in both these test systems suggested that, like other anti-estrogens, the action of tamoxifen was due to a high association constant but low effectiveness of the complex it formed with estrogen receptors (i.e., it was a partial agonist, with high affinity but low intrinsic activity)[35]. This was a pharmacological action with which ICI researchers had become familiar in their work on the beta-blockers (Quirke, 2006). It helped to cast further light on the physiological processes at a molecular level[36], and made tamoxifen a particularly useful research tool for investigations of hormone-dependent tumors (Jordan et al., 1972).

Rendered confident by the clinical and laboratory studies carried out so far, Walpole's team began planning trials in contraception, and the Nolvadex Development Programme was drawn up[37]. This would play an important part in the drug's transformation from quasi-orphan to blockbuster drug (Quirke, 2012b).

[28]It was thought that British gynecologists would be unlikely to come across more than one woman a year to whom abortion could be offered under the new law. Walpole et al. (AZ CPR 101/19B Endocrinology and Fertility, 19 Feb. 1971).

[29]Walpole et al. (AZ CPR 101/17B Endocrinology and Fertility 25 June 1970).

[30]Richardson, "The History of Nolvadex."

[31]Ibid.

[32]Walpole et al. (AZ CPR 101/18B Endocrinology and Fertility, 28 Oct. 1970). Norethisterone was a drug with such an androgenic effects. See Richardson, "The history of Nolvadex."

[33]Walpole et al. (AZ CPR 101/17B Endocrinology and Fertility 25 June 1970).

[34]Walpole et al. (AZ CPR 101/19B Endocrinology and Fertility 19 Feb. 1971).

[35]Tamoxifen is now classed as a selective estrogen receptor modulator (SERM), meaning that it activates estrogen receptors in some tissues, while blocking them in others.

[36]Walpole et al. (AZ CPR 101/18B Endocrinology and Fertility 28 Oct. 1970); idem, (AZ CPR 101/19B Endocrinology and Fertility 19 Feb. 1971).

[37]AZ PH 19597 B: Nolvadex Development Programme, June 1971.

The Nolvadex Development Programme (1971)

The "Development Programme" was an organizational innovation which standardized and codified the R&D process at ICI. It marked the transition from the "Proving Trial" to the "Development Trial Stage[38]," thus helping to bring together the "R" and the "D" in R&D[39]. ICI's first Development Programme had been written up in 1964 for the beta-blocker propranolol (Inderal)[40]. It followed a series of quarterly development reports[41], and coincided with the hitherto separate Research and Development Departments coming together under the responsibility of a single Director, the Technical Director, as well as with the creation of the CSD in 1963. It therefore was a response to both internal and external factors and stimuli.

The Nolvadex Development Programme, which came 7 years after the Inderal Development Programme, included 16 rubrics, describing the work done up to June 1971 (the date of the start of the Programme), making an assessment of the drug's potential market, and plans for future work:

1. Clinical trials
2. Further laboratory work
3. Analysis
4. Sales formulation
5. Packaging
6. Process development and manufacturing of bulk drug
7. Manufacture and packing of tablets
8. Position in North America
9. Launch dates
10. Registration
11. Competitive situation
12. Sales estimates
13. Trade Mark and approved name
14. Patents
15. Publications
16. R&D costs

Three important considerations were taken into account when planning future work. First and foremost were tamoxifen's possible clinical uses, based on the results of trials received to date. These included: treatment of estrogen-dependent mammary carcinoma; induction of ovulation on women suffering from infertility due to failure to ovulate; menstrual disorders associated with abnormal levels of endogenous estrogen; oral contraceptive (a) for women, (b) for men; treatment for oligospermia; test for pituitary function; others. Secondly, the drug's position in North America was under question, following Ayerst's rejection of ICI's offer of Nolvadex for the American market, and the FDA's likely negative attitude toward its use in breast cancer. This attitude may have been due to a 1971 report in *JAMA* which had suggested that there was a link between diethylstilbestrol and a rare form of vaginal cancer, and was promptly followed by an FDA bulletin warning against the use of DES (FDA, 1971). Thirdly, the commercial situation, shown in **Table 1**, indicated that a number of therapeutic treatments of hormone-dependent breast cancers were already in existence, each of which commanded almost equal shares of the market.

TABLE 1 | UK major branded products.

	Annual sales (NHS level)	Cost of 1 week's treatment
Provera 100 (Progestogen, Upjohn)	£50,000	£3.15
Masteril (Anabolic/androgen, Syntex)	£45,000	£1.35
Deca-durabolin (Anabolic/androgen, Organon)	£35,000	£0.75
Durabolin (Anabolic/androgen, Organon)	£28,000	£0.50
SH420 (Progestogen, Schering)	£27,000	£0.70

Source: AZ PH 19597 B: Nolvadex Development Programme (June 1971).

Despite such competition from rival firms in America and Europe, tamoxifen had two advantages on which its market position would ultimately depend in relation to breast cancer: (1) its unique mode of action in being an estrogen-antagonist without androgenic properties, and since at the time it was the only product of its type its use should be larger; (2) it possessed very low incidence of side-effects compared with other forms of treatment. Another important consideration was that of past R&D costs (shown in **Table 2**), which had a bearing on budgeting and planning for future expenditure.

The gaps in particular columns and rows in **Table 2** exemplify the non-linear nature of pharmaceutical R&D, with bottle necks and feedback loops when advances in one area are held up by, and then develop in response to those in another. They also illustrate the pivotal part played by drug regulation in shaping the research and development activities of pharmaceutical firms. The trials that followed the CSM's approval for Nolvadex in 1969 not only led to an increase in existing expenditure in areas such as biochemistry, but to new expenditure in areas such as formulation (shown in bold).

As well as further trials in anovulatory infertility (in Abderdeen, Oxford, London, and Dublin), and in breast cancer (Manchester, Glasgow, and London), the Nolvadex Development Programme included plans for trials in contraception. "In view of the reluctance of British gynecologists" to become involved in such trials, in 1971 ICI contacted Professor Egon R. Diczfalusy, co-founder and Director of the WHO Research and Training Centre on Human Reproduction at the Karolinska Institute in Stockholm[42], where he had already carried out collaborative

[38] "Minutes of a meeting of the ICI 46,474 Development Team, Development Department, held on 28th August 1970" (Appendix 4 of Richardson, "The history of Nolvadex").

[39] AZ PH 19597 B: Nolvadex Development Programme, June 1971.

[40] AZ PH15355B: Inderal Development Programme, 15 June 1964.

[41] AZ CPR 68B: Research and Development Quarterly Reports (1962-1964).

[42] Ian Askew, "Obituary: HPR co-founder, Egon R. Diczfalusy, 1920–2016," http://www.who.int/reproductivehealth/obituary-e-diczfalusy/en/ (accessed 02.06.17).

TABLE 2 | Nolvadex R&D costs (£'000).

	1964	1965	1966	1967	1968	1969	1970	1971	Est 1972
Biology	6.7	1.7							
Toxicology	9.6	9.5	10.0	2.2	1.7			**1.1**	
Biochemistry	1.8	1.7	2.7		2.4	7.2	**8.3**	**8.0**	**2.0**
Analytical	1.8		0.2	0.9	0.4	0.2	**1.5**	**5.0**	**3.0**
Formulation							**0.4**	**4.8**	**5.0**
Process development	1.9			0.1			**5.0**	**14.7***	
Medical				0.5	0.7	2.8	**3.5**	**5.6**	**8.0**
Development				0.5	0.4	0.5	**1.0**	**1.7**	**2.0**
Totals	21.8	12.9	12.9	4.2	5.6	10.7	**19.7**	**40.9**	**20.0**

**Including cost of bulk drug.*
Source: AZ PH 19597B: Nolvadex Development programme (June 1971).

projects involving healthy human volunteers using estrogens and other compounds[43].

The Swedish trials led to the finding that, contrary to what might be expected from the laboratory studies in rats, tamoxifen stimulated rather than suppressed ovulation, and therefore would not work as a contraceptive pill in women. The market for a fertility drug was small, as seemed the market for an anti-cancer drug, partly due to the poor prognosis associated with the disease. Despite growing clinical evidence of the usefulness of tamoxifen in breast cancer, the very low sales estimates produced by the Marketing Department suggested that it was never going to cover the R&D costs and bring an appropriate return to the company. ICI's Main Board therefore made the decision to close down the Programme, but tamoxifen's champion, Walpole, threatened to resign. On this announcement, despondency spread through the entire research department. Moreover, when informed of the company's decision, one clinician said that, in view of the encouraging trial results, ICI could not *morally* withdraw the drug[44]. By then, the breast cancer trials had led to a number of publications, which sparked world-wide interest in tamoxifen[45]. Under such pressure, the company reversed its decision, Walpole remained, and the project was saved. In February 1973 ICI applied for a product license, which was granted a few months later, and in October of that year Nolvadex was launched in the UK for both anovulatory infertility and the palliative treatment of breast cancer. Although there continued to be crossovers between the two projects, the rest of this paper will focus on breast cancer. It will show how tamoxifen was transformed from a research object and palliative therapy for advanced breast cancer, into a diagnostic and predictive tool, an adjuvant chemo-endocrine treatment first in post-menopausal, then also in pre-menopausal women with early breast cancer, and eventually into the first chemopreventative for cancer.

Tamoxifen, from Palliative Care to Adjuvant Therapy (1973–75)

Among the large number of clinical trials now being carried out with tamoxifen, Dr. Einhorn's studies at the Karolinska Institute in Stockholm had included a measurement of the rate of DNA synthesis in breast tumors and the effect this had on treatment. As a result, his group had been able to anticipate clinical response to, or relapse after, treatment with tamoxifen. From these observations, Walpole concluded that tamoxifen could be employed in pre-menopausal women with breast cancer for a short period as a tool to predict the usefulness of drastic treatments such as ophorectomy in these women. At the same time, he began making plans for a trial with Dr. J. C. Heuson of the European Breast Cancer Group, who was anxious to compare tamoxifen with Nafoxidine (an Upjohn compound which like tamoxifen could bind to the estrogen receptor, but unlike tamoxifen had several toxic side effects). The trial would include estrogen receptor determinations on biopsies taken from each patient to determine whether there was a correlation between clinical response to the compound and the presence of estrogen receptors in the tumor tissue[46]. By then, the clinical trials in fertility and contraception had also shown that in some instances tamoxifen led to the suppression of lactation. Walpole felt that this action would be of interest in the context of breast cancers which may be associated with high blood prolactin levels, and indeed at Westminster Hospital two patients who had responded well to tamoxifen had tumors which were thought to be prolactin-dependent[47]. Taken together, these observations on the measurement of DNA synthesis before and after treatment, of the content of estrogen receptors in breast tumors, and of blood prolactin levels led to the hope that it would be possible to predict the type of patient likely to respond to treatment with tamoxifen, i.e., to develop what is now referred to a "stratified therapy" (i.e., a re-branding of what was formerly known as "personalized medicine"; Smith, 2012).

However for this to happen, better screens had to be devised, first in animals and then in humans. In her unpublished history of tamoxifen, Dora Richardson commented that no laboratory tests for anti-tumor activity had been carried out with tamoxifen until *after* its activity in patients had been confirmed. The laboratory model adopted by Walpole's team to test for tumor inhibition was the DMBA (dimethyl benzanthracene) induced tumor in rats (also known as the Huggins tumor). The next step was to design a simplified method of receptor analysis, which could be applied routinely on a large scale in this model, before being applied in humans[48]. Walpole's team developed such a method in collaboration with Craig Jordan (from the Department of Pharmacology at Leeds University, who at the time was on leave of absence at the Worcester Foundation for Experimental Biology, USA, and whose work would later be sponsored by ICI; (Jordan, 2006), pp. 40–41). If it proved effective, i.e., if it demonstrated that tamoxifen could bind to the estrogen

[43] Walpole et al. (AZ CPR 101/20B, 28 June 1971). On the WHO Research Centre see: http://ki.se/en/kbh/who-center-for-human-reproduction (accessed 02.06.17). See also Oudshoorn (1998).
[44] Richardson, "The History of Nolvadex"; see also Jordan (2003).
[45] Walpole et al. (AZ CPR 101/26B Fertility, 22 June 1973).
[46] Ibid.
[47] The presence of prolactin in human blood had been confirmed by Henry Friesen et al. (1970).
[48] Walpole et al. (AZ CPR 101/27B Fertility 24 Oct. 1973).

receptor in human breast tumors, it was hoped that this method would make it possible to screen patients for the presence of specific estrogen receptor in biopsy specimens of their tumors and to pre-select for treatment with Nolvadex those in whom such receptors had been found. However, alongside these highly scientific methods, clinicians continued to use observations such as "hot flushes" as indications that the treatment was working and remission was likely to occur[49]. Walpole therefore proposed that physiological indicators might also be used to ensure that individual patients were not being "under-treated" and could be given the maximum effective dose to produce an improved response[50].

In his report of February 1974, Walpole wrote: "By good fortune, Nolvadex was launched at a time of increased interest in the assessment of the endocrine status in breast cancer[51]." Tamoxifen was shown to be highly effective in binding to the estrogen receptor and, before long, researchers in Europe and the US were therefore using tamoxifen as a tool to "predict the response of breast tumors to hormone therapy[52]." However, this new use for tamoxifen brought out the fact that not all patients whose tumors had demonstrable estrogen receptor levels responded well to endocrine therapy. Although this paradox might be due to the fact that the receptor assays used were not of consistent standard, it suggested that a number of biochemical events were a pre-requisite for complete endocrine regulation, and that other lesions occurred in patients for whom endocrine therapy failed, thereby casting further light upon the complex processes involved in malignant disease.

Nolvadex was also launched at a time when the value of chemotherapy in cancer was being established with novel drugs tested first alone, then combined, in collaborative multi-center trials (see Keating and Cambrosio, 2007; also Quirke, 2014, pp. 670–671). With drug resistance becoming a growing concern, not only in bacteria, but also in cancer cells, combination therapy was being developed and its modalities refined. Hence, in June 1974, Walpole began planning a trial in which two different treatment modalities, supposedly devoid of cross-resistance, would be used, and he proposed to alternate their administration on a 4-week basis[53]. The rationale for this trial was that, unlike conventional sequential treatments, each alternating treatment would be started *before* rather than *after* the effect of the previous one was exhausted, thus resulting in a cumulative effect. Two added benefits of such an approach were that (1) drugs with high levels of toxicity, such as adriamycin and vincristin, could be given for much longer, and (2) at precise moments in the treatment cycle, the patient's bone marrow and immune system would have a chance to recover. This approach was tried by Dr. Heuson under the aegis of the European Organization for Research and Treatment of Cancer (EORTC, which had been created in 1962), alongside another trial in pre-menopausal women[54].

Such plans and discussions, which were based on a growing number of publications and symposia presenting evidence not only of symptom relief, but also of remissions and survival from breast cancer[55], indicate that, both as a research tool and a therapeutic agent, tamoxifen was shifting from palliative care into the realm of chemotherapy, transforming it in the process. What follows will concentrate on the years 1975–1980, after which ICI's research reports on tamoxifen and related topics ended. During that period Walpole was mainly involved in the Nolvadex Development Programme until his sudden death in 1977. Although his involvement ensured continuity between the research and development phases, Walpole's gradual disengagement from the research, which can be detected in the reports, meant that the project lacked clear purpose and direction. Months were lost to pressures of competing work inside the company, and aspects of the research were outsourced to external laboratories (Jordan, 2006, Chapter 3). Nevertheless, in that time, the foundations were laid for the next phase in tamoxifen's trajectory, from adjuvant therapy to the first chemopreventative remedy for cancer.

1975–1980: The Final Years of ICI's Tamoxifen Project

Clinical trials carried out in Britain by Ward (Birmingham) and Brewin (Glasgow) and beyond (in Germany) showed that the response to tamoxifen in patients who experienced a recurrence of their breast tumor after primary surgery and/or radiotherapy tended to increase with age[56]. These findings prompted the question of what the mechanism for this action might be, since tamoxifen was an "anti-estrogen." Could it be that tamoxifen exerted an estrogenic action (albeit a weak one) by way of its metabolites?[57] The study was taken up at ICI by Barry Furr[58] and B. Valaccia, and a programme of synthesis and tests of analogs of tamoxifen metabolites in a number of different screens, not only estrogen, but also progesterone and androgen receptor screens, was initiated to find out whether tamoxifen could bind with them, and therefore be useful in other cancers. Later prostaglandin synthetase (PGS) inhibitor screens were also developed by the team. These showed that tamoxifen was an effective inhibitor of human breast tumor PGS in addition to arresting tumor growth, thus offering an explanation for the clinical observation that patients taking Nolvadex for advanced breast cancer often experienced relief from bone pain, and strengthening the rationale for its use

[49] Walpole (AZ CPR 101/28B Fertility 26 Feb. 1974).
[50] Walpole (AZ CPR 101/28B Fertility 16 June 1974).
[51] This interest was stimulated by observations such as Jense's (Jense et al., 1971), who showed that patients with tumors containing high affinity estrogen receptors were more likely to have a remission following adrenalectomy than those without such receptors (with remissions in 10/13 patients with positive tumors, but in only 1/26 patients without).
[52] Walpole et al. (AZ CPR 101/32B Fertility 27 June 1975). One of these researchers was W. L. McGuire in the USA. See McGuire et al. (1975).
[53] Walpole (AZ CPR 101/28B Fertility 14 June 1974).
[54] Walpole (AZ CPR 101/32B Fertility 27 June 1975). For more on the history of EORTC see: http://www.eortc.org/history/ (accessed 05.06.17). Its journal, the *European Journal of Cancer,* was launched in 1965.
[55] Bonadonna et al. "Cytotoxic chemotherapy for mammary cancer," Symposium, Padova, 8 Apr. 1974, quoted in Walpole (AZ CPR 101/28B Fertility 16 June 1974).
[56] Walpole (AZ CPR 101/32B Fertility 27 June 1975).
[57] Gregory (AZ CPR 101/34B Fertility 17 Feb. 1976).
[58] "Professor Barry Furr" Obituary: https://alumni.reading.ac.uk/our-alumni/dr-barry-furr (accessed 10.06.17).

in adjuvant chemotherapy further still. Hence, it was hoped as a result of this programme that a follow-up compound for Nolvadex might be found—the target being an anti-estrogen of similar potency to Nolvadex with one or more of the following properties in addition: lower agonist activity, shorter half-life, greater inhibitory activity against PGS, anti-androgenic activity[59].

This new research strand, which would lead ICI to its second major breakthrough in cancer therapy: ICI 118,630 (goserelin, Zoladex), was stimulated by the discovery by Schering Plough of the first non-steroidal anti-androgen Flutamide for the treatment of prostate cancer. As they had done earlier with Merrell's drug, ICI therefore mobilized their synthetic capabilities and the scientific expertise acquired with tamoxifen to search for a non-steroidal anti-progestin (which unlike anti-androgens would have the advantage of having neither anti-anabolic activity nor any effects on "normal sexual behavior")[60]. Another approach consisted in looking for a novel, potent analog of the luteinizing hormone-releasing hormone (LHRH), also referred to as the gonadotrophin-releasing hormone (GnRH), although this was initially expected to be used mainly in animal breeding[61]. As well as testing the compounds in the company's by now well established receptor-binding assays, once again the team needed to develop new *in vivo* screens, and "in view of the previous experience with Nolvadex, that is anti-estrogenic in the rat and estrogenic in mice," tests would have to be carried out in more than one species. Because the chick comb was known to be androgen sensitive and chicks were cheap, it was chosen as one of the animal models in which to test active compounds and compare them to Flutamide.

Meanwhile, a special organization had been created for the purpose large-scale clinical studies of tamoxifen as an adjuvant treatment for cancer: the Nolvadex Adjuvant Trial Organisation (NATO). Until then, adjuvant therapy had consisted either in chemotherapy using mainly cytotoxic drugs, or in major endocrine ablation after curative surgery. Clinical trials of tamoxifen in adjuvant therapy therefore began in 1976, some progressing ahead of schedule, and their favorable results, which showed that Nolvadex was effective in both pre- and post-menopausal women regardless of their receptor status, were frequently discussed at symposia and in the medical press from 1977 onwards[62]. Not only did these results change the modalities of adjuvant therapy for breast cancer whilst helping to establish tamoxifen in the treatment of the early stages of the disease (NATO, 1983, 1988), but in the context of these adjuvant trials evidence also emerged of the drug's potential to *prevent* the recurrence of breast cancer in women at high risk (i.e., who had already had cancer in one breast). This potential was explored in a trial carried out in Denmark, with the aim of establishing the value of tamoxifen as a "prophylactic" in breast cancer (Andersen et al., 1981; Mouridsen et al., 1988). Patients were selected who had had a mastectomy with or without radiation and in whom there was no evidence of metastases, for it was known that 55–60% of them would develop local recurrence of the disease or metastases within 5 years. They were then randomly allocated either to Nolvadex, stilboestrol, or a placebo[63]. The trial eventually showed that although 10% of the women treated with placebo developed a recurrence of their breast cancer, none of those treated with tamoxifen had experienced such a recurrence[64]. Such results would later help to justify the initiation of breast cancer prevention trials, for instance the Breast Cancer Prevention Trial NSABP-P1 (BCPT), with the aim of establishing whether 5 years of tamoxifen would reduce the incidence of invasive breast cancer in women identified as being at high risk of the disease, and yet healthy (Fosket, 2010; Löwy, 2012; also Fosket, 2004).

Almost as soon as it had moved into the realm of cancer chemotherapy, tamoxifen therefore hinted at the theoretical and practical possibilities of chemoprevention in cancer. Further trials would turn tamoxifen into the first preventative for any cancer, helping to establish the broader principles of chemoprevention, while extending the market for tamoxifen and similar drugs further still (Early Breast Cancer Trialists Collaborative Group, 1992, 1998)[65].

Tamoxifen, from the Clinic into the Medical Marketplace

Thanks to tamoxifen, ICI were able to tap into the vast cancer research network connected in Europe through the EORTC, and across the Atlantic through the National Cancer Institute (NCI). The interest tamoxifen generated among scientists and clinicians, rather than the promotional activities of the company, which Dora Richardson argued remained very limited, greatly enhanced its position in the medical marketplace. In a personal communication to Walpole, Dr. Scott Lippman of the NCI had described his method for testing tamoxifen in human breast cancer cell lines which were dependent on estrogens for their long-term growth in tissue culture[66]. In these cells, tamoxifen showed itself to be strongly inhibitory of both DNA and protein synthesis. Lippman had turned this method into a "kit" for measuring receptors, and spurred by their American subsidiary (ICI-USA), ICI did not waste time in starting work on their own quantitative assay "kit to be marketed as an adjunct to Nolvadex." Such a kit would not only make money for itself, but by helping to justify the use of tamoxifen, would further enhance the market position for the drug, particularly in the USA[67]. By then, the company had submitted an Investigational New Drug (IND) application to the Food and Drug Administration (FDA). It was followed in 1976 by a New Drug Application (NDA) to the FDA's Oncological Drugs Advisory Committee, in which John Patterson, a member of ICI's Clinical Research Department (formerly of the Medical Department), made a detailed and

[59] Crossley (AZ CPR 101/37B Fertility 27 Jan. 1977).
[60] Ibid.
[61] Ibid.
[62] Richardson, "The history of Nolvadex."
[63] Walpole (AZ CPR 101/32B Fertility 27 June 1975).
[64] Richardson "The history of Nolvadex".
[65] NB: the FDA approved tamoxifen for the reduction of breast cancer risk in 1998.
[66] Walpole, (AZ CPR 101/32B Fertility 27 June 1975).
[67] Crossley (AZ CPR 101/33B Fertility 2 Oct. 1975). Such kits, which would now be referred to as "companion diagnostic tests," remain a mainstay of breast cancer therapy today.

convincing case for Nolvadex in breast cancer[68]. By 1984, the NCI were describing tamoxifen as the adjuvant chemotherapy of choice for breast cancer (Consensus Conference, 1985). Although ICI's application for a US patent for tamoxifen had originally been rejected on the basis that the US Patent Office did not recognize advances on existing inventions, and that Merrell's patent for clomiphene pre-dated that for tamoxifen, in 1985 the American court of appeals finally granted ICI the patent rights for tamoxifen in the USA, thereby starting the 17-year patent cover there, paradoxically at a time when it was coming to an end in other countries (Jordan, 2006, p. 40).

Tamoxifen's entry into the American market contributed to rising worldwide sales: although ICI's Marketing Department had only expected it to make £100,000 p.a. in 1970, by 1974 figures on the home market alone amounted to £140,000, overtaking one of ICI's well established drugs Mysoline (for epilepsy). By 1976, sales figures were equivalent to those for the anesthetic Fluothane, the first drug to put ICI's Pharmaceutical Division "in the black," and for over-the-counter drugs such as, the antiseptic Savlon. As the expiry date for their tamoxifen patents was drawing near, in 1979 ICI obtained a 4-year extension for their UK patent, on the basis of "the nature and merits of the invention in relation to the public," as well as "the profits made by the patentee[69]." By 1980, it was making £30 M for the firm[70].

Nevertheless, even as late as September 1982, at the annual portfolio review attended by the managers of the Biology Department (Dr. J. D. Fitzgerald) and Chemistry (Dr. R. Clarckson), the manager of the Marketing Department, who also attended the meeting, commented that "there was no market for cancer[71]." ICI's Marketing Department were not alone in under-estimating the market for anti-cancer drugs: if tamoxifen had not been "stolen" by American companies while it remained unprotected by patents, it was partly because they did not believe in its usefulness either (Jordan, 2006, p. 40). The fate of tamoxifen therefore rested on the qualities of the drug itself, and the interest it generated not only among researchers both inside and outside the company, but also among patients and the wider public. As mentioned earlier in this paper, Dora Richardson's history of Nolvadex was—quite unusually for such an internal publication—accompanied by letters from patients who attributed their lives to tamoxifen. Appendix 6 entitled "What do the patients think" included a letter to ICI's Pharmaceutical Division, in which a grateful patient wrote: "Thank you for a miracle."

Tamoxifen benefited not only from being the first of a kind, which helped to confer upon it the status of a "miracle drug," but once again from its origins as a contraceptive pill. As the name indicates, it could be taken orally, and this mode of administration meant that Nolvadex was suitable for home treatment, and a large proportion of sales (75%) occurred through retail pharmacies. This enabled local tinkering with established protocols, as well as a degree of self-experimentation, as testified by another letter, written by a cancer researcher (Dr. June Marchant of the Regional Cancer Registry, West Midlands Oncology Group), who had been diagnosed with breast cancer, and having spent 20 years in cancer research was well versed in the modalities of cancer therapy[72]. After discussing her ideas with her clinician, whom she described as "understanding," together they worked out "an unconventional management programme." Because her thymus gland was within the radiation field of her breast tumor she refused radiation therapy. Instead, she decided to undergo therapy with a new cytotoxic drug that was being tested locally in a clinical trial. She appeared to make an uneventful recovery, but in 1972 a scan revealed metastases in her brain. At this point, she therefore elected local treatment with radiation of the head and adjuvant therapy with tamoxifen. Knowing from her own research that prolactin had been identified as a hormone with perhaps an even greater significance than estrogen in the maintenance of breast tissue and breast tumor growth, she started reading the relevant literature. A number of inhibitory substances had been tried on a few patients with breast cancer, and among them Levodopa appeared to give beneficial results. Her clinician therefore agreed to give her Levodopa as additional anti-hormonal therapy. Her drug regimen was phased out in 1975, and at the time of writing her letter, in 1976, the author felt "very well indeed, having had no ablative operation, cytotoxic drugs or masculinizing hormones." From her own experience, she therefore concluded that "systemic therapy, in addition to local therapy, had a vital role to plan in the management of the disease," and she wished to share this positive experience with others.

Her conclusions went beyond ascertaining the value of tamoxifen in adjuvant therapy—extrapolating from her experience with the drug, she defended "systemic therapy" more generally. Yet, after Dr. Stephen Carter, who had been responsible for ICI's cancer project on Cell Division and Growth[73], left the company, taking early retirement in 1979, he was not replaced, and the project on cell growth was terminated. Thus, in 1980, when tamoxifen was bringing in sizeable profits for the company and Zoladex (for prostate cancer) was in the pipeline, ICI had no longer a cancer research programme, a situation that lasted until in 2006, when Alderley Park became the Global Lead Centre for the company's cancer research[74].

DISCUSSION

If tamoxifen made it into the medical marketplace, it was largely *despite* rather than because of the company's marketing

[68] Richardson, "The History of Nolvadex."

[69] UK 1949 Patents Act, Section 23. http://www.legislation.gov.uk/ukpga/1949/87/pdfs/ukpga_19490087_en.pdf (accessed 15.06.17). I thank Dr. Michael Jewess for pointing out this section to me.

[70] Richardson, "The History of Nolvadex."

[71] Dr. J.D. Fitzgerald, personal communication.

[72] Dr. June Marchant, "Personal view" (27 November 1975), Appendix 6 of Richardson, "The History of Nolvadex."

[73] AZ CPR 110 (1965-77): Cell Division and Growth.

[74] On Alderley Park, see AstraZeneca, "Alderley Park, Cheshire," http://www.pharmaceutical-technology.com/projects/astrazeneca-alderley/ (accessed on 15.06.17). This has become Manchester's new bioscience campus: https://mspl.co.uk/campuses/alderley-park/ (accessed on 15.06.17), while AZ's UK R&D center has been relocated to Cambridge see: https://www.astrazeneca.com/our-science/cambridge.html (accessed 15.06.17).

department. Thanks to having inside a drug champion prepared to risk his career to save his project and a medical department willing to run the gauntlet of the FDA to promote tamoxifen in the USA, but also thanks to interest generated outside, among scientists, clinicians, and patients who asked for or agreed to take the drug, it was transformed from a failed contraceptive pill into a successful breast cancer medicine. The patients' letters referred to in this essay provide us with a unique insight into this transformation, but also into the public demand and experimentation which escape the control of both the industry and the professions, and are not normally included in discussions of pharmaceutical innovation.

Focusing on the early history of tamoxifen has made it possible to examine in some detail both the brakes and the stimuli for pharmaceutical innovation. These come from inside as well as outside industry, contrary to a rather narrow model of pharmaceutical innovation, according to which companies, motivated by a commercial more than a scientific agenda, push drugs onto an unsuspecting public, often with the connivance of the medical profession, but hopefully kept in check by the actions of regulatory authorities (for example see: Crawford, 1988; Marsa, 1997; Law, 2006).

In the case of tamoxifen, pharmaceutical innovation was predominantly science- and clinic-driven, rather than market-driven (so a case of demand-pull rather than supply-push, Walsh, 1994). It benefited from a number of coincidences: its ability to bind to the newly-discovered estrogen receptor helped to make it into a useful tool for investigating hormone-dependent tumors, as well as a drug of choice for treating breast cancer. It was developed at a time when palliative care was becoming an important part of cancer treatment (Clark, 2007), and when chemotherapy was successfully being applied to cancer in collaborative trials. These placed ICI at the center of a global network of cancer institutions and organizations, which helped to maintain interest in their drug even as ICI was losing its research focus on cancer. Hence the last phase in tamoxifen's transformation, into the first chemopreventative for cancer, owed more to this global network than to ICI's efforts at promoting their drug. Finally, tamoxifen was developed at a time when cancer patients were encouraged to demand better treatments, to become more proactive in their own care, and engage with ideas of risk.

In the beginning, when tamoxifen was being developed as a contraceptive pill, cancer patients had to some extent been used as "proxies" for normal, healthy human subjects, and their voices were mostly heard trough the clinicians who reported on their symptoms as indications of the drug's activity and side-effects. Nevertheless, the fact that their voices were included, both indirectly in the reports and directly in Dora Richardson's history of Nolvadex, suggests that to the company these voices did matter: they helped to shape the content of the research, whilst justifying it, both morally and scientifically. Rather than a "detour" in relation to contraception (Oudshoorn, 2002), the study of tamoxifen in breast cancer was therefore carried out in close parallel with its study in contraception (and subsequently fertility). This is not surprising, given that ICI's interest in cancer pre-dated their interest in contraception by 20 years. Nevertheless, the contraception project helped to determine tamoxifen's fate as a drug: from what it was (a synthetic anti-estrogen, safe with a relatively low incidence of side-effects), to how it could be taken (orally, and therefore suitable for home treatment).

Thus, the drug and its fate were shaped by the industrial setting from which it emerged. In return, tamoxifen transformed the biomedical landscape in which it was deployed. As it moved from contraception into cancer, tamoxifen expanded its market at the same time as its clinical role, transforming cancer therapy in the process. Not only did it cast fresh light on the function of sex hormones and their role in malignant disease, but it hinted at the possibility of personalized medicine, and helped to lay the foundations of chemoprevention. Indeed, although the concept of chemoprevention had already begun to take hold with drugs to treat cardiovascular diseases (to lower cholesterol or blood pressure, for instance), by becoming associated with and tapping into the drive to catch cancer early by screening and, even better, prevent it by introducing life-style and other changes, the principles and practice of chemoprevention were further strengthened by drugs like tamoxifen and their application to the field of cancer.

In the context of cancer chemoprevention, the question of its use in normal, healthy women arose once more, but it did not go unchallenged. In her chapter on "Breast Cancer Risk as Disease", Jennifer Fosket has described the controversies that surrounded the BCPT which took place in the USA in the 1990s, highlighting the fact that the risks associated with tamoxifen were often downplayed, and this despite letters from ICI (which had spun off its pharmaceutical division to form Zeneca) warning both doctors and women enrolled on the trials that—by then—some women taking tamoxifen had developed endometrial cancer (Fosket, 2010, p. 345). Although the BCPT identified an increased risk of pulmonary embolism, deep-vein thrombosis, as well as endometrial cancer in women who had taken tamoxifen compared to the control group on placebos, their findings were nonetheless favorable to tamoxifen: only 124 women had developed breast cancer in the tamoxifen group, compared to 244 in the placebo group (Fisher et al., 1998). On the other hand, the results of the Royal Marsden Study, carried out in the UK at roughly the same time as the BCPT, were not so clear-cut: they revealed no significant reduction in breast cancer incidence in women at risk who took tamoxifen (Powles et al., 1998). These different results were attributed to key differences between the American and European trials, ranging from their organization, to the numbers of women enrolled, the criteria for their selection, and different conceptualizations of what constituted "high risk[75]." Such differences and controversies surrounding the trials led the FDA to downgrade its approval from "prevention" to "reduction of risk" (Fosket, 2010, p. 348).

In a sense then, tamoxifen had been the victim of its own success. Originally intended for women with little chance of

[75] Foskett has suggested that Royal Marsden selected women based on their family history, and this may have led to more women with the BRCA1 and 2 gene mutations, for whom tamoxifen is a less effective preventative, being enrolled in the trial (Fosket, 2010, n. 2 p. 352).

survival, its ability to cause disease in women experiencing long-term remissions thanks to tamoxifen led to a complex assessment of risk, which had to be shared with women undergoing treatment for breast cancer. Thus, in 1996, a guide written for clinicians and patients on the subject of tamoxifen stressed the importance of communicating the risks involved in taking the drug, from minor side effects such as hot flushes, to potentially serious ones including other cancers. Hence, what was nevertheless a message of hope did not only relate to tamoxifen itself, but also to the "new patient" which caring professionals, breast cancer advocates, and the media had helped to create: "prepared with background information about the disease"; requiring "treatment options"; wanting "good communication and information" and wanting "the truth" (Langer, 2006, p. 134). Such patients did exist, as we saw in the case of June Marchant, even though she may have been exceptional, and in many ways drugs like tamoxifen had also helped to bring them about.

CONCLUDING REMARKS

The focus of this paper on the industrial context for the development of tamoxifen highlights the importance of the early phases in the history of pharmaceutical innovation, for this early history shapes the form and content of drugs, has the potential to define their use and ultimately determine their fate in the medical marketplace, and this despite the many twists and turns that characterize their trajectory from bench to bedside.

This particular focus also throws into sharp relief the contribution made by applied research to the advancement of scientific knowledge: in the case of tamoxifen, more specifically to the understanding of basic physiological processes involved in human reproduction and malignant disease. Such a contribution is in part due to the fact that industry, perhaps more easily than academia with its rigid disciplinary boundaries, enables a to-ing and fro-ing between separate, yet contiguous research projects and therapeutic areas (in this instance, between contraception, fertility, and cancer). This to-ing and fro-ing between projects illustrates once again the non-linear nature of pharmaceutical innovation. Typified by blind alleys, fresh departures, feedback loops between the laboratory and the clinic, as well as serendipitous discoveries, the early history of tamoxifen brings to the fore the role of human agency, the institutional memory that is often associated with long-term investment in particular areas of expertise, and is embodied in individual researchers like Walpole.

Just as the industrial context is worthy of historical enquiry, the early history of drugs such as, tamoxifen, which are at once emblematic and idiosyncratic examples of pharmaceutical innovation, may yield useful lessons for potential innovators, by helping them to identify key moments when choices are made and decisions taken, so that these may in time be revisited and alternative paths may be explored. For innovators are at once the makers and the products of history, even if history is often remote from their concerns or absent from their writings. Unfortunately, because of the growing difficulty of accessing pharmaceutical archives, this rich vein of historical enquiry may fast be coming to an end. The hope remains that, as an essential component of their intellectual capital, such archives will continue to be available to researchers both inside and outside companies.

TOPIC EDITORS' DECLARATION

This article is classified as "Original Research" as it reports on primary sources of a historical nature, including previously unpublished studies.

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REVIEW
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Addressing the Challenges of Tuberculosis: A Brief Historical Account

Hussam W. Al-Humadi[1,2]*, *Rafal J. Al-Saigh*[1] and *Ahmed W. Al-Humadi*[2]

[1] Department of Pharmacology and Toxicology, Pharmacy College, University of Babylon, Babylon, Iraq, [2] Laboratory of Pharmacology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Tuberculosis (TB) is a highly contagious disease that still poses a threat to human health. *Mycobacterium tuberculosis* (MTB), the pathogen responsible for TB, uses diverse ways in order to survive in a variety of host lesions and to subsequently evade immune surveillance; as a result, fighting TB and its associated multidrug resistance has been an ongoing challenge. The aim of this review article is to summarize the historical sequence of drug development and use in the fight against TB, with a particular emphasis on the decades between World War II and the dawn of the twenty first century (2000).

Keywords: tuberculosis, history, treatment, anti-TB drugs, multidrug resistance, pharmaceutical innovation

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***Correspondence:**
Hussam W. Al-Humadi
alhumadi2010@gmail.com

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INTRODUCTION

Tuberculosis (TB) is a very old infectious disease, caused by *Mycobacterium tuberculosis* (MTB) (Dye and Williams, 2010). It's still the second most frequent cause of death in the world (WHO, 2014), reaching up to 10 million new cases every year (Dye and Williams, 2010); more interestingly, latent cases represent one third the world's population (WHO, 2014), with 10% of latent TB cases to progress to active infection (Selwyn et al., 1989), especially in diabetic or human immunodeficiency virus (HIV) positive patients, or those undergoing an immunotherapy (Barry et al., 2009). Active TB is characterized by chronic cough with bloody sputum, night sweats, fever and weight loss, while other organs (apart from the lungs) can be infected and cause a wide range of symptoms (Dolin Gerald et al., 2010).

TB bacilli are spread with the droplets of respiratory secretions that are associated with cough or sneezing of the infected person. The MTB can then invade and replicate within the endosomes of the pulmonary alveolar macrophages (Houben et al., 2006; Kumar et al., 2007) leading to clinically active disease in about 10% of cases (Dye et al., 1999; WHO, 2009), while further growth of the remaining cases can be arrested by a competent immune response. However, in those with arrested cases, the bacilli are completely eradicated in about 10% of the individuals, with the remaining 90% entering a dormant or latent state in which there is a containment of the infection. As pathogens escape from the microbicidal action of the host immune cells (phagosome-lysosome fusion; MHC class I, class II, and CD1 molecules antigens; nitric oxide and other reactive nitrogen intermediates), latent TB and the dormant bacilli are reactivated with any serious disruption (decline) in the host immune state (HIV infection, diabetes mellitus, renal failure, chemotherapy and immunosuppressive therapy, malnutrition, etc.) that occurs (Dye et al., 1999; Corbett et al., 2003; Frieden et al., 2003; Wells et al., 2007; Dooley and Chaisson, 2009; WHO, 2009).

The unique clinical manifestations of MTB are attributed to the high lipid content of this pathogen (Southwick, 2007); the latter has an outer membrane lipid bilayer (Niederweis et al., 2010) and therefore, hematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain, and even the bones (Harries, 2005; Herrmann and Lagrange, 2005; Kumar et al., 2007).

The public health challenge of TB has been managed by a number of drugs and treatment strategies over the years, but this challenge has always been much bigger in certain parts of the world. The spreading of the HIV infection has been a major factor in managing the TB challenge, and so has been the increasing resistance of MTB strains to the high efficacy first line anti-TB drugs (**Table 1**; WHO, 2009) which leads to the growing incidences of drug resistant strains: multiple drug resistant (MDR) and extensively drug resistant (XDR). These strains pose a significant threat, especially for immunocompromised patients who are significantly less likely to recover without the assistance of effective drugs. Other factors that may contribute in disease progression include poverty, population expansion, active transmission in overcrowded places (hospitals, prisons, and other public places), migration of individuals from high-incidence countries due to wars or famine, drug abuse, social decay, homelessness (Frieden et al., 2003; Hill et al., 2004; Mathema et al., 2008) and technical problems like poor quality of detection, in addition to health status (old age, malnutrition, and medical conditions that compromise the immune system) (Corbett et al., 2003; Frieden et al., 2003; Wells et al., 2007; Dooley and Chaisson, 2009).

Furthermore, the unusual structure and chemical composition of the MTB cell wall (which hinders the entry of drugs and leads to drugs resistance) (Brennan and Nikaido, 1995) as well as the capability of the MTB cell to lie dormant at a low metabolic rate, in a deep location in pulmonary cavities or inside solid material that makes antibiotic penetration difficult. Finally, expensive, long-term therapy, disturbed therapeutic regimens, dosage variance and irregularity in follow up, form additional challenges for an effective TB management (Lawn and Zumla, 2011).

The aim of this review article is to summarize the historical sequence of drug development and use in the fight against TB, with a particular emphasis on the decades between World War II and the dawn of the twenty first century (2000).

TABLE 1 | Classification of anti-tuberculosis (anti-TB) drugs according to WHO (2010).

Lines	Grouping	Drugs	
First-line anti-TB drugs	Group 1 (oral)	Isoniazid (H/INH)	
		Rifampicin/rifampin (R/RIF)	
		Pyrazinamide (Z/PZA)	
		Ethambutol (E/EMB)	
		Rifapentine (P/RPT)	
		Rifabutin (RFB)	
Second-line anti-TB drugs	Group 2 (injectable)	Aminoglycosides	Streptomycin (S/STM)
			Kanamycin (KM)
			Amikacin (AMK)
		Polypeptides	Capreomycin (CM)
			Viomycin (VIM)
	Group 3 (oral and injectable; fluoroquinolones)	Ciprofloxacin (cfx)	
		Levofloxacin (lfx)	
		Moxifloxacin (mfx)	
		Ofloxacin (OFX)	
		Gatifloxacin (GFX)	
	Group 4 (oral)	Para-aminosalicylic acid (PAS)	
		Cycloserine (DCS)	
		Terizidone (TRD)	
		Ethionamide (ETO)	
		Prothionamide (PTO)	
		Thioacetazone (THZ)	
		Linezolid (LZD)	
Third-line anti-TB drugs	Group 5 (oral and injectable)	Clofazimine (CFZ)	
		Linezolid (LZD)	
		Amoxicillin plus clavulanate (AMX/CLV)	
		Imipenem plus cilastatin (IPM/CLN)	
		Clarithromycin (CLR)	

HISTORICAL SEQUENCES IN MANAGEMENT OF TB: BEFORE WORLD WAR II

There is evidence of TB being present in humans since antiquity (Lawn and Zumla, 2011). MTB has been detected in the remnants of a bison in Wyoming that lived 17,000 years ago (Rothschild et al., 2001), while researchers have found tubercular decay in the spines of Egyptian mummies (3000–2400 BC) (Zink et al., 2003), and genetic studies suggested TB was present in America since around 100 AD (Konomi et al., 2002). In Europe, TB had begun to rise between seventeenth and nineteenth century, in which it reached a peak level and caused about 25% of all deaths (Bloom, 1994). At that time, several measures had been taken including the improvement of life style and the encouragement of the infected people to enter sanatoria (McCarthy, 2001). However, 50% of those who entered sanatoria died within 5 years (McCarthy, 2001).

On 24 March 1882, MTB was identified and described by Robert Koch; he was later honored with the Nobel Prize (1905) for this discovery (Nobel Foundation, 2014), the "TB World Day" was established on that date. Koch didn't pay attention for the similarity between bovine and human TB, therefore, the recognition for TB-infected milk as a way of TB transmission was delayed until the invention of the pasteurization process, that reduced it dramatically. Koch announced a glycerin extract of the TB bacilli as a "remedy" for TB in 1890, calling it "tuberculin." Even though, it was not effective, it was later adapted as a screening test for the presence of latent TB (Waddington, 2004).

In 1906, Albert Calmette and Camille Guérin achieved the first genuine success in immunization against TB by using attenuated bovine-strain TB. It was called the "bacille Calmette-Guérin" (BCG). This vaccine was first used on humans in 1921 in France (Bonah, 2005), but the vaccine got widespread acceptance in the US, Great Britain, and Germany only after World War II (Comstock, 1994).

The discovery of penicillin initiated the war against various infectious microorganisms, and has set the basis for a greater motivation to discover other antibacterial and antimicrobial compounds for overcoming diseases like TB. The success of penicillin during World War II pushed researchers to study other molds (Aminov, 2010), one of them being Streptomyces griseus, found in chickens; as a result, streptomycin was successfully purified in 1943 and used as an anti-TB therapy in 1945 (Schatz et al., 1944; Kerantzas and Jacobs, 2017). Unfortunately, the overuse of streptomycin led to the development of drug-resistance (Kerantzas and Jacobs, 2017), but the end of World War II saw major developments in pharmacology been established, and a number of drugs being developed and used against TB (**Figure 1**; Schatz et al., 1944; Wassersug, 1946).

HISTORICAL SEQUENCES IN MANAGEMENT OF TB: AFTER WORLD WAR II

In 1946, the Medical Research Council (MRC) TB Unit in the UK was established, and a clinical study designed for comparing streptomycin with bed rest vs. bed rest alone (Marshall, 1949) was launched. As expected, high clinical improvement was seen in streptomycin with bed rest in comparison to bed rest alone, however, a greater improvement was seen in the first 3 months, and many patients deteriorated later on due to the emergence of streptomycin resistance (Kerantzas and Jacobs, 2017).

Better results followed with the development of para-aminosalicyclic acid (PAS), which was an oral agent (unlike streptomycin) and could be used in combination with streptomycin (Lehmann, 1946; British Medical Journal, 1950; Fox et al., 1999; Williams, 2009). In 1950s, several anti-TB drugs with different mechanisms of action were discovered and developed, including PAS, isoniazid, pyrazinamide, cycloserine and kanamycin (**Figure 1**, **Table 2**). In 1951, streptomycin plus isoniazid were introduced as a TB therapy (Fox et al., 1999), while rifampicin (in 1960) allowed the shortening of TB therapy to 9 months when given with isoniazid, and to 6 months when given with pyrazinamide (American Thoracic Society, 2003). By the 1970s, five antibiotics were available against TB (**Figure 1**). Afterwards, the MRC TB Unit developed the current short-course therapeutic regimen (isoniazid, rifampicin, pyrazinamide and ethambutol) in collaboration with the United States Public Health Service.

Latent TB has been treated usually with a single antibiotic to prevent progressing to active TB disease (Menzies et al., 2011), while active TB is now treated with combinations of antibiotics in order to reduce the growing risk of antibiotic resistance (Lawn and Zumla, 2011). Directly observed therapy-short course (DOTS) is currently recommended by the WHO as an effort to reduce the number of people not appropriately taking antibiotics (Volmink and Garner, 2007; Liu et al., 2008; Mainous and Pomeroy, 2010). When MDR-TB is detected, treatment with at least four effective antibiotics for 18–24 months is recommended (Lawn and Zumla, 2011). A person with fully-susceptible MTB may develop secondary resistance because of inadequate therapy, or using low-quality medication (O'Brien, 1994).

DRUG RESISTANCE FOR TB

More than 50% of the world's MDR-TB cases are found in India and China, where about 5.4% of MDR-TB cases progress to XDR-TB (WHO, 2010). The MDR-TB treatment is a combination of 8–10 drugs for 18–24 months (Gandhi et al., 2010). Resistance to the two most effective first-line anti-TB drugs, rifampicin and isoniazid, is known as MDR-TB, while resistance to three or more of the six classes of second-line drugs is known as XDR-TB (**Table 1**; CDC, 2006); the latter has been identified in more than 90% of the world's

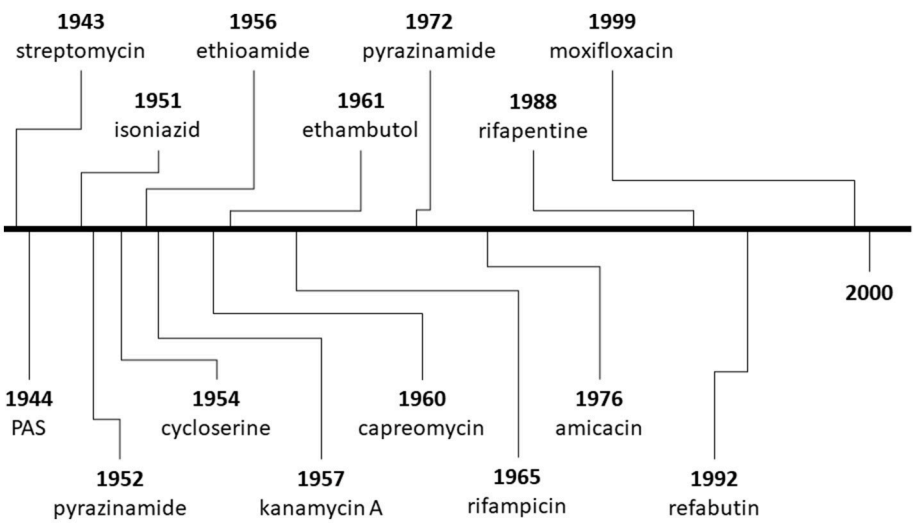

FIGURE 1 | Timeline of the discovery of major compounds used for the treatment of tuberculosis (TB) from the Second World War until 2000.

TABLE 2 | Classification of anti-tuberculosis (anti-TB) drugs according to the site of action and its respective mechanism of resistance.

Drug classification	Groups	Anti-TB drugs	Mechanism of action	Target TB	Efficacy	Mechanism of resistance	References
Cell envelope synthesis inhibitor	Peptidoglycan	Cycloserine	Inhibit 2 enzymes forming D-alanine residues	MDR, XDR	High	Mutations in *alrA*	Patel et al., 2012
		Terizidone	Cycloserine derivative	MDR, XDR	High	Non	Galietti et al., 1991
	Arabinogalactan	Ethambutol	Inhibiting arabinosyltransferase, arabinose acceptor	Active	Low	*embCAB* operon	Telenti et al., 1997; Wolucka, 2008
	Mycolic acid	Isoniazid	Activation katG enzyme and inhibits inhA gene	Active, latent	High	Mutations in *katG* and *inhA* genes	Vilche'ze and Jacobs, 2007; Riccardi et al., 2009
		Triclosan	Inhibits the inhA enzyme without activation katG	MDR	Low	–	Wang et al., 2004; Freundlich et al., 2009
		Pyridomycin	Inhibits the inhA enzyme	MDR, XDR	High	Mutations in *inha*	Hartkoorn et al., 2012
		Ethionamide	Inhibits InhA by enzyme ethA	MDR, XDR	High	Mutations in *inha*, *etha*	Wolff and Nguyen, 2012
		Prothionamide	Inhibits InhA by enzyme ethA	MDR, XDR	High	Mutations in *etha*	Wang et al., 2007
		Thiocarlide	Inhibiting synthesis of oleic acid	MDR, XDR	High	Mutations in *ethA*	Phetsuksiri et al., 2003
		Delamanid	Releasing Nitric oxide by Ddn enzyme	Active, latent, MDR, XDR	High	Non	Gler et al., 2012; Zhang et al., 2013
		SQ109	Membrane transporter *MmpL3*	Active, latent	High	Non	Owens et al., 2013
Protein synthesis inhibitor	Aminoglycosides	Streptomycin	Bind to 30S subunit of ribosome	MDR, XDR	High	Mutation in *rpsl*	Honort and Cole, 1994
		Amikacin	Bind to 30S subunit of ribosome	MDR, XDR	High	Mutation in *rrs*	Sowajassatakul et al., 2014
		Kanamycin	Bind to 30S subunit of ribosome	MDR, XDR	High	Mutation in *rrs*	Sowajassatakul et al., 2014
	Oxazolidone	Linezolid	Bind to 50S subunit of ribosome	MDR, XDR	High	Mutation in *G2576T*(23S)	Scheetz et al., 2008
		Sutezolid	Bind to 50S subunit of ribosome	MDR, XDR	High	Non	Zumla et al., 2015
	Peptidoglycan	Capreomycin	Peptidoglycan Breakdown	MDR, XDR	High	Mutation in *tlyA*	Chen et al., 2003
Nucleic acid inhibitor	Rifamycins	Rifampicin	RNA polymerase inhibitor	Active, latent	Low	Mutations in *rpoB* gene	Sensi, 1983; Telenti et al., 1993
		Rifapentine	RNA polymerase inhibitor (b-subunit)	Active, latent/HIV	High	Mutations in *rpoB* gene	Chan et al., 2014
		Rifabutin	RNA polymerase inhibitor (b-subunit)	Active, latent/HIV	High	Mutations in *rpoB* gene	Yan et al., 2015
		Rifalazil	RNA polymerase inhibitor (b-subunit)	Active, latent/HIV	High	Mutations in *rpoB* gene	Saribaş et al., 2003
	PAS	PAS	Folic acid synth inhibitor	MDR, XDR	High	Mutations in *thyA*	Patel et al., 2012
	Quinolones	Levofloxacin	DNA gyrase inhibitor	MDR, XDR	High	Mutations in *gyrA*	Pranger et al., 2011
		Moxifloxacin	DNA gyrase inhibitor	MDR, XDR	High	Mutations in *gyrA*	Pranger et al., 2011
New drugs		Bedaquiline	Inhibiting ATP synthase enzyme	Active, MDR, dorment,XDR	High	Mutations in *atpE* gene	Chan et al., 2013; Chahine et al., 2014
		Pyrazinamide	Interferes with binding to mRNA	Active,MDR	High	Mutations in *RpsA, pncA*	Zhang et al., 2003; Shi et al., 2011
		Clofazimine	Inhibits DNA replication	MDR, XDR/HIV	High	Mutations in *rv0678*	Arbiser and Moschella, 1995

PAS:para-aminosalicylic acid.

countries (Akachi et al., 2012). Total drug-resistant to all currently used drugs (McKenna, 2012) was first observed in Italy (2003) (Migliori et al., 2007), and had also been reported in Iran and India (Velayati et al., 2009; Akachi et al., 2012), but not widely reported until 2012 (WHO, 2006; Migliori et al., 2007).

MTB strains undergo spontaneous mutations that lead to resistance of one or more anti-TB drug (David, 1970). Thus, the exposure of MTB population to a single anti-TB drug could inhibit its growth but not completely eradicate it, therefore, regrowth and mutations leading to progressive drug resistance, these mutated genes are eventually triggering continuous proliferation of the bacilli and recurrence of symptoms, which is called "the fall and rise phenomenon" (Espinal, 2004). Hence, low drug levels due to insufficient drug bioavailability or from malabsorption (e.g., in HIV patients) have emerged in the etiology and mechanism of anti-TB drug resistance. Furthermore, continuous use of old anti-TB regimens may not target specific populations of MTB under certain circumstances that hardly act in acidic, or hypoxic conditions within caseous foci or inside macrophages (Mitchison, 1998). The resistance also developed independently for each drug in combined anti-TB regimens at a specific time through mutation processes.

We now know that MDR and XDR-TB infections' danger can be overcome by preventing resistance of already sensitive anti-TB drug within the combination regimen (Morris et al., 1995). As some studies reported, the resistance arises from replicating bacilli, while non-replicating bacilli do not undergo mutation and no resistance can be developed. Thus, minimizing the drug resistance can be performed by extending the therapeutic duration and subjecting MTB to drugs with longer half-lifes (Gumbo et al., 2009). Some newest agents that are used for MDR-TB, such as bedaquiline, are tentatively recommended (Chahine et al., 2014). Ineffective and inadequate anti-TB treatment could fail to achieve goals in about 30% of MDR-TB patients (Mitnick et al., 2003). The treatment of XDR-TB is very difficult, because XDR-TB bacilli are resistant for more drugs other than isoniazid and rifampicin, including fluoroquinolones and aminoglycosides (Ma et al., 2010).

The drug-resistant TB can be predicted in TB patients with unsuccessful therapy (relapse) or those who are in close contacts with MDR-TB patients (Becerra et al., 2011). Therefore, a 5 month treatment with positive sputum smear or culture is closely attributed to MDR-TB strains (Lew et al., 2008), and in such cases, several molecular methods for diagnosis of MDR-TB are enrolled, including the Xpert MTB/RIF, which is currently available for the detection of rifampicin resistance (Menzies et al., 2009; Sharma et al., 2015).

The last two decades have witnessed an ongoing effort to understand the molecular bases for anti-TB resistance and to further investigate the genetic traits in MDR- and XDR-TB strains (Nachega and Chaisson, 2003). Mutated genes that associated with MDR- and XDR-TB are described in **Table 2**, which are classified as first line anti-TB drugs resistance, that starts with isoniazid resistance; the latter is connected to alterations in the catalase-peroxidase gene (katG), the inhA gene, which encodes in an enzyme involved in mycolic acid biosynthesis (Vilche'ze and Jacobs, 2007; Riccardi et al., 2009). Rifampin resistance, including its derivatives (rifapentine, rifabutin and rifalazil) resistance, is associated with genetic mutations in rpoB, which encodes the RNA polymerase β-subunit (Sensi, 1983; Telenti et al., 1993; Saribaş et al., 2003; Chan et al., 2014; Yan et al., 2015). Pyrazinamide resistance is linked to mutations in pncA, that eliminates the pyrazinamidase/nicotinamidase activity (Zhang et al., 2003; Shi et al., 2011). Ethambutol resistance is conferred to genetic mutations with the embCAB operon, which facilitates production of arabinosyl transferase (Telenti et al., 1997; Wolucka, 2008). In spite of the role of the second line drugs to overcome the MDR that linked with the first line drugs, second line drugs are also linked with genetic mutations like the first line agents: streptomycin resistance which is associated with mutations in the rpsL, ribosomal S12 protein, and rrs, 16S rRNA gene (Honort and Cole, 1994); kanamycin and amikacin resistance are closely linked to genetic mutations of streptomycin (Sowajassatakul et al., 2014); while capreomycin resistance is attributed to mutagenesis of the tlyA gene, which has homology to rRNA methyltransferases (Chen et al., 2003). Quinolones resistance (like levofloxacin and moxifloxacin) is associated with mutation of gyrA gene encoding DNA gyrase (Pranger et al., 2011). Ethionamide resistance is linked to inhA mutations, in addition to cross-resistance between isoniazid and ethionamide in mutations of the etaA (ethA) gene, which is responsible for ethionamide activation (Wolff and Nguyen, 2012). The resistance to PAS is linked to mutations within the thyA gene, which produces thymidylate synthase A (Patel et al., 2012), while cycloserine resistance is conferred with activation of the alrA gene as D-alanine racemase encoding, which causes increased over expression of alrA (Chacon et al., 2002).

Advances in MTB targeting have emerged through the exploration of the genome sequence of MTB (Cole et al., 1998), but unfortunately this approach gave little success (Payne et al., 2007) as it is not predicting the drug ability of the discovered new agent (Working Group on New TB Drugs, 2010). Genome sequencing of MTB, identification of the essential signaling and metabolic pathways, assessment of physicochemical properties of the MTB and other methods are still employed in order to discover newer agents with high specificity and less toxicity with good efficacy. In parallel, reengineering and repositioning of the old known drugs have been adapted to achieve better results in therapy, but the challenges of the resistance still threaten this goal and the discovering of the new agents remain the main approach to counteract the deterioration in situation over the world (Koul et al., 2011).

IMMUNOMODULATORY AND REPURPOSING DRUGS AGAINST TB

An efficient and competent host immune system is crucial for the eradication of an MTB infection and/or containment of latent TB infection (Migliori and Huggett, 2009; Zumla et al., 2012; Wallis et al., 2013). The stability of latent TB state is achieved by MTB ability to attenuate and evade host mycobactericidal

responses. Inadequate immunity leads to MTB multiplication and clinical symptoms' development. Acceleration of the host inflammatory response may lead to tissue destruction; therefore, several agents are being used in order to manipulate and reduce the destructive inflammatory responses, or augment protective immunity to enhance recovery and minimize the duration of therapy (Subbian et al., 2011; Tobin et al., 2012).

In experimental animals, the role of pro-inflammatory and anti-inflammatory eicosanoids in the process of regulating tumor necrosis factor-α levels (Tobin et al., 2012) and in tailoring TB treatment, depends on the host genotype (Skerry et al., 2012). Administered prophylactically or therapeutically, the ABL family tyrosine kinase inhibitor, such as imatinib, reduced the MTB load and the granulomatous lesions in MTB-infected organs and was also effective against a rifampicin-resistant strain of MTB when co-administered with current first-line TB drugs (Napier et al., 2012). Furthermore, using generic, non-steroidal anti-inflammatory (NSAIDs) and analgesic drugs as an adjunct therapy in experimental animal models, has a wide clinical distributed (Ivanyi and Zumla, 2013). NSAIDs can reduce MTB load and alleviate lung damage in mice (Vilaplana et al., 2013), and they show anti-TB activity in phenotypical assays (Guzman et al., 2013).

Both verapamil (a calcium-channel blocker) and reserpine (an adrenergic neuron blocking agent) have efflux pump inhibitory properties that could decrease macrophage-induced drug tolerance (Amaral et al., 2007; Adams et al., 2011); as a result, both could be added to anti-TB regimen to decrease the duration of curative therapy. Ivermectin is an anti-nematode agent that also has bactericidal activity against MTB (Lim et al., 2013). Cilostazol and sildenafil—as phosphodiesterase inhibitors—could be added to the anti-TB regimen, as they improve the resolution of tissue pathology, accelerating MTB clearance and diminishing therapeutic period (Maiga et al., 2012). Lansoprazole, a well-known proton-pump inhibitor, was also found to be effective against intracellular MTB by targeting its cytochrome bc1 complex through intracellular sulfoxide reduction (metabolite enzyme) to lansoprazole sulfide; this metabolite enzyme is crucial for the bacterium to produce energy, thereby killing it off (Rybniker et al., 2015). Metformin, which is a drug used for the treatment of type 2 diabetes, acts as inhibitor to a mitochondrial complex which is similar to bacterial NDH complex, thus enhancing the targeting of an anti-TB drug toward intracellular MTB (Cole et al., 1998; Vashisht and Brahmachari, 2015). Finally, chemical and biological immunomodulatory agents have also been evaluated to accelerate host immune responses in anti-TB therapy (Uhlin et al., 2012), with MDR-TB cure rate enhancement, prevention of recurrence and shortening therapy duration occurring as a result.

TB/HIV CO-INFECTION

It is known that the concomitant use of anti-retroviral therapy (ART) with the treatment of drug-susceptible pulmonary TB improves survival rates in HIV-infected individuals. However, treatment of TB in such patients is complicated, due to potential drug interactions and the risk of developing "immune reconstitution inflammatory syndrome" (Gengiah et al., 2011). The important drug interactions occur between the rifamycins and the protease inhibitors as well as non-nucleoside reverse transcriptase inhibitor drugs. Rifamycin derivatives (rifampicin, rifabutin and rifapentine) induce liver enzymes and reduce serum concentrations of protease inhibitors, such as indinavir, nelfinavir, saquinavir, ritonavir, amprenavir, atzanavir, and fosamprenavir. Rifabutin is the least potent inducer of CYP3A (Weber et al., 2001) and rifapentine falls in between rifampicin and rifabutin in its capacity to induce CYP3A. Rifapentine is not recommended for the treatment of TB in HIV-infected individuals because of the increased rate of acquired rifamycin resistance (Dheda et al., 2010). Rifabutin is used as a substitute for rifampicin in the treatment of active TB in patients receiving ART. On the other hand, delaying initiation of ART until TB treatment is completed in HIV-infected individuals significantly increases mortality across the spectrum of immunodeficiency. Clinical trials have reported that early ART in TB patients co-infected with HIV decreases mortality (Havlir et al., 2011). The World Health Organization recommends that ART should be started within the first 8 weeks of initiating TB treatment (Blanc et al., 2011; De Cock and El-Sadr, 2013), while the optimal timing of initiating ART in patients with TB-HIV co-infection in Sub-Saharan Africa remains an urgent research priority (De Cock and El-Sadr, 2013).

PREVENTION

The prevention and control of TB depend primarily on vaccination of infants and appropriate diagnosis and treatment of active cases (Lawn and Zumla, 2011). The US Preventive Services Task Force (USPSTF) recommends screening high risk people for latent TB with either tuberculin skin tests or interferon-gamma release assays (Bibbins-Domingo et al., 2016). The only available vaccine since 1921 is BCG (McShane, 2011). In children, BCG decreases the risk of getting the infection by 20% and the risk of infection turning into disease by nearly 60% (Roy et al., 2014). It is the most widely used vaccine worldwide, with more than 90% of all children being vaccinated (Lawn and Zumla, 2011). However, it should be noted that the immunity induced by the vaccine decreases after about 10 years (Lawn and Zumla, 2011). Moreover, as TB is uncommon in most of Canada, the UK, and the USA; BCG is administered only to those at high risk (CDC, 2006; Teo and Shingadia, 2006; Public Health Agency of Canada, 2010). Finally, the drawback of the BCG vaccine is making the tuberculin skin test result false positive; therefore, this test not widely used in screening for TB anymore (Teo and Shingadia, 2006).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium

Luca Falzone[1], *Salvatore Salomone*[1,2]* *and Massimo Libra*[1,2]

[1] Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy, [2] Research Center for Prevention, Diagnosis and Treatment of Cancer (PreDiCT), University of Catania, Catania, Italy

The medical history of cancer began millennia ago. Historical findings of patients with cancer date back to ancient Egyptian and Greek civilizations, where this disease was predominantly treated with radical surgery and cautery that were often ineffective, leading to the death of patients. Over the centuries, important discoveries allowed to identify the biological and pathological features of tumors, without however contributing to the development of effective therapeutic approaches until the end of the 1800s, when the discovery of X-rays and their use for the treatment of tumors provided the first modern therapeutic approach in medical oncology. However, a real breakthrough took place after the Second World War, with the discovery of cytotoxic antitumor drugs and the birth of chemotherapy for the treatment of various hematological and solid tumors. Starting from this epochal turning point, there has been an exponential growth of studies concerning the use of new drugs for cancer treatment. The second fundamental breakthrough in the field of oncology and pharmacology took place at the beginning of the '80s, thanks to molecular and cellular biology studies that allowed the development of specific drugs for some molecular targets involved in neoplastic processes, giving rise to targeted therapy. Both chemotherapy and target therapy have significantly improved the survival and quality of life of cancer patients inducing sometimes complete tumor remission. Subsequently, at the turn of the third millennium, thanks to genetic engineering studies, there was a further advancement of clinical oncology and pharmacology with the introduction of monoclonal antibodies and immune checkpoint inhibitors for the treatment of advanced or metastatic tumors, for which no effective treatment was available before. Today, cancer research is always aimed at the study and development of new therapeutic approaches for cancer treatment. Currently, several researchers are focused on the development of cell therapies, anti-tumor vaccines, and new biotechnological drugs that have already shown promising results in preclinical studies, therefore, in the near future, we will certainly assist to a new revolution in the field of medical oncology.

Keywords: cancer, antineoplastic drugs, chemotherapy, targeted therapy, cell therapy

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Apostolos Zarros,
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Reviewed by:
Yanqi Ye,
University of North Carolina at Chapel Hill, United States
Darius John Rowland Lane,
Florey Institute of Neuroscience and Mental Health, Australia

***Correspondence:**
Salvatore Salomone
salomone@unict.it

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INTRODUCTION

Epidemiology of Cancer

Cancer is often referred to as the "Pathology of the Century" assuming the connotations of an endemic disease spread throughout the world. It has also been defined as the "the modern disease par excellence" (Roy Porter) or even the "the quintessential product of modernity" (Siddhartha Mukherjee) (Bynum and Porter, 2005; Mukherjee, 2011; Arnold-Forster, 2016). These two definitions are universally recognized and are justified by the drastic increase in incidence and mortality, witnessed since the end of the eighteenth century until today, where cancer represents the second leading cause of death worldwide (Ferlay et al., 2015). In particular, in 2015, over 8.7 million cancer deaths were recorded worldwide and about 17.5 million new cases of neoplasia were diagnosed (GBD Mortality Causes of Death Collaborators, 2016). Moreover, despite advances in the diagnostic, medical and interventional fields, the number of new cases of cancer has increased by about 33% in the decade 2005–2015, mainly due to the increase in population and the increase in the average age of life. Conversely, mortality rates are almost unchanged, although many Countries experienced a decrease in cancer mortality notwithstanding increasing incidence rates (Global Burden of Disease Cancer Collaboration et al., 2018).

Supporting these recent epidemiological data, the examination of a longer period of time and the analysis of the data collected by the National Cancer Institute (NCI) over the last 40 years showed that there has been a continuous and almost stable increase in incidence rates of all cancers. While a general decrease in mortality rates was recorded above all in the last 20 years, although from the period between 1975 and 1995 there was a slight increase in mortality rates (**Figure 1**). The reduction in mortality rates can be easily associated with the continuous progress in the medical and pharmacological fields that has allowed to reduce the cancer deaths, thanks to the recent introduction in therapy of more effective drugs and therapeutic approaches (Soneji et al., 2014; Miller et al., 2016).

When epidemiological data concerning the so-called "big killers" (i.e., cancer of lung, breast, colon, prostate, stomach, liver, cervix uteri, esophagus, bladder, non-Hodgkin's lymphoma (NHL), pancreas, melanoma) are examined, it is instead observed how the trend of incidence and mortality rates are very variable according to the type of pathology taken into account. In particular, some tumor types, such as lung cancer and pancreatic cancer, have still maintained mortality rates almost unchanged compared to 40 years ago (**Figure 1**).

These epidemiological data show that, even nowadays, cancer represents a global health problem and one of the greatest challenges in the medical field, despite the important pharmacological and therapeutic discoveries we have seen since the second post-war period up to the present day (Gittelman, 2016).

Cancer Treatment Before the 1900s

As previously mentioned, cancer is considered a modern disease, but the oncology has their roots in much older times, as evidenced by antique documents dating back to ancient Egyptian and Greek civilizations (Sudhakar, 2009).

Several historical and scientific records show that cancer was present even before the appearance of human on earth. In fact, in some fossil remains of dinosaurs or prehistoric animals traces of bone tumors have been found, probably osteosarcomas or bone metastases (Rothschild et al., 2003; Dumbravbrevea et al., 2016). The first historical and scientific records of tumors in humans date back to the Egyptian period, around 3000 B.C., and refer to the writings contained in a papyrus found by Edwin Smith, in which a case of breast cancer and the surgical treatment adopted were described (Breasted, 1930; Sanchez and Meltzer, 2012).

Other writings date back to 1500 B.C.; in particular, the Ebers' papyrus, contains information on different types of cancer (skin cancer, uterine cancer, stomach cancer and rectum), where the neoplastic pathology is recognized as incurable. In this papyrus, cancer is not considered as due to physical or biological causes, but, rather, as the results of esoteric forces, linked to the negative will of the ancient Egyptian Gods (Ebers, 1875; Bryan and Smith, 1930; Kelly and Mahalingam, 2015).

It was only in 400 B.C. that cancer was recognized as a disease with specific biological causes rather than related to supernatural events. In particular, the first "scientist" describing cancer in a scientific way was Hippocrates, who considered the tumor as a disease caused by the imbalance between the 4 main body humors, i.e., blood, phlegm, yellow and black bile. Furthermore, Hippocrates enunciated the first scientific theory on the origins of cancer, hypothesizing that it originated when there was an excess of black bile in the body, and divided the tumors into three different categories: hard cancers, ulcerated cancers, and hidden cancers, defining the latter incurable (Karpozilos and Pavlidis, 2004; Tsoucalas and Sgantzos, 2016).

The theory of body humors remained predominant for a long time and was further refined by Galen, a physician who lived in Rome around the 130–200 A.C. Galen improved the Hippocratic theory by defining tumors as curable pathologies when caused by the alterations of yellow bile, and incurable when caused by the alterations of the black bile, particularly when this last humor infiltrates the tissues. Furthermore, Galen indicated the first surgical strategies for the treatment of tumors (Galen, 1565; Papavramidou et al., 2010; Hajdu, 2016).

From the Ancient Greek and Greco-Roman Age up to the Modern Era the theories of Hippocrates and Galen remained the most credited. Moreover, from the Egyptian period until the end of the nineteenth century the treatment of cancers was mostly based on the adoption of a healthy diet, cautery and radical surgical approaches for superficial tumor forms, while for the incurable deep forms, mostly used palliative pain therapies, based on poppy (*Papaver somniferum*) extract (Faguet, 2015). The use of these antiquated therapeutic practices often hesitated in the death of the patient, due to the progression of the very same tumor and/or to sequelae of infectious nature related to the surgical intervention and the poor hygienic conditions.

We may consider modern oncology as born in the 1700s, when scientists began to study, for the first time, the carcinogenic effects of some substances, such as tobacco or soot. However, a

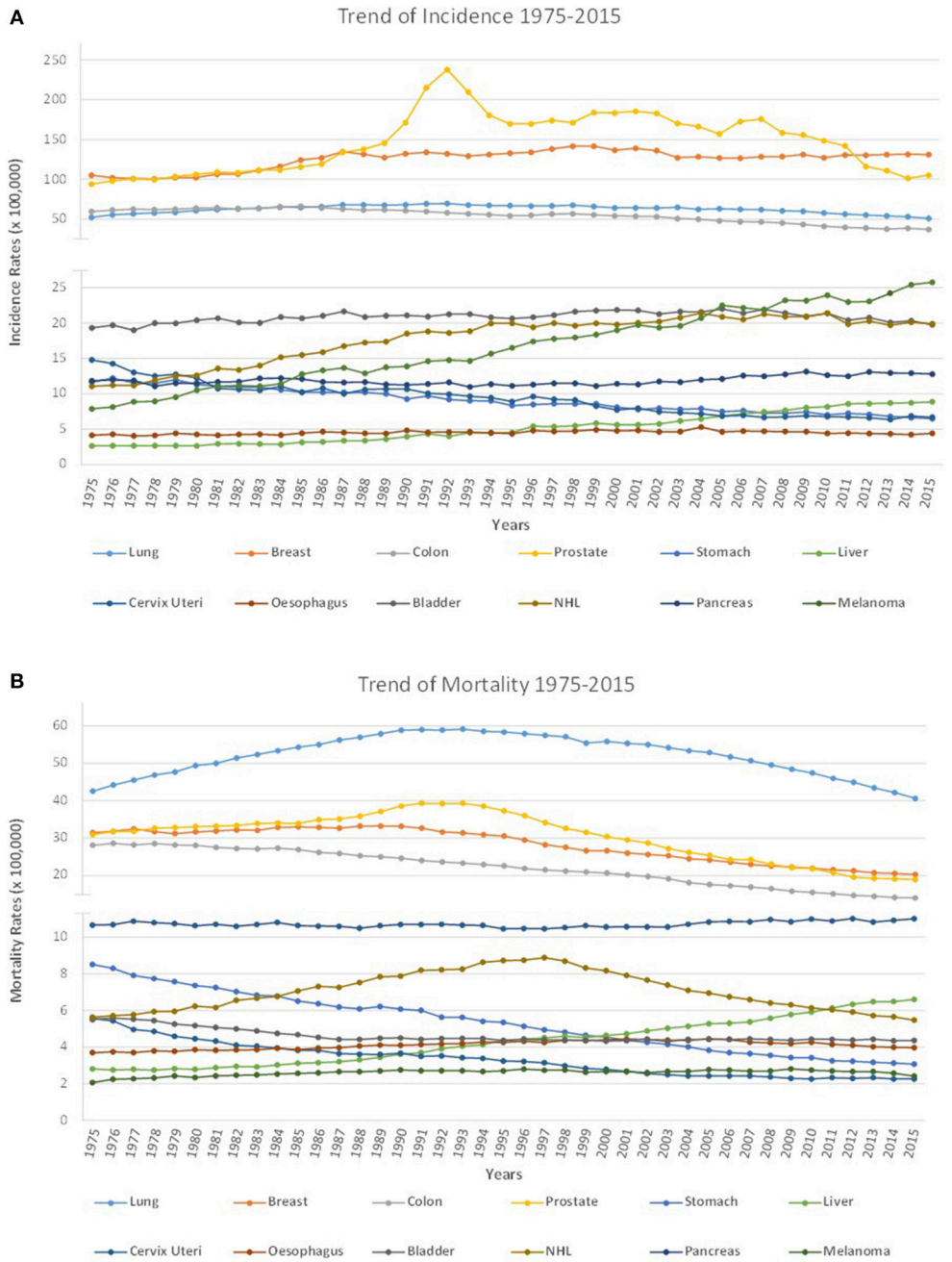

FIGURE 1 | Cancer incidence and mortality from 1975 to 2015. **(A)** In the last 40 years, from 1975 to 2015, there has been a general increase in incidence rates for several cancers as a consequence of the demographic increase. Thanks to cancer prevention and screening strategies the number of many types of cancer has decreased. In particular, for cervix uteri and stomach cancers, a decrease in incidence rates was observed, while the incidence rates for esophagus, bladder, lung and colon remained unchanged. Finally, an increase in incidence rates was recorded for hepatic carcinoma, for breast cancer, NHL and above all for melanoma, whose incidence has increased radically in recent years; **(B)** Cancer mortality rates have globally decreased thanks to the development of targeted therapy and immunotherapy. In particular, in the last 40 years there has been a decrease in mortality rates for prostate, breast, colon, bladder, and especially for cervix uteri and stomach cancers. Almost unaltered mortality rates are observed for melanoma, pancreatic and esophageal cancers for which really effective pharmacological treatments are not yet available. An increase in mortality was observed for hepatic carcinoma while lung cancer and NHL showed a variable trend over the years with a slight increase in mortality rates from 1975 to 1995, and a general decrease in mortality rates recorded above all in the last 20 years.

number of previous important discoveries and inventions paved the way for the birth of modern oncology. From the sixteenth century to the late nineteenth century, there has been a revolution in the medical, surgical and interventional field thanks to the important discoveries of many scientists who studied tumors from an anatomical, biological, epidemiological and therapeutic

point of view. This revolution began with the discoveries of Paracelsus (1493–1541) to reach the intuitions of Percival Pott (1714–1788), passing from the invention of the microscope and the theories of cancer onset postulated by Rudolf Virchow (1821–1902), to the first approaches of experimental oncology and radiotherapy promoted by the first medical oncologists and by Marie and Pierre Curie, respectively (Faguet, 2015; see **Table 1** for the milestones of oncology before 1900).

In particular, the origin of radiotherapy dates back to the late XIX century, with the discovery of X-rays by Wilhelm Conrad Röntgen (Röntgen, 1896; Busacchi, 2015). In the following years, Marie and Pierre Curie identified a substance with radiations two million times higher than Uranium (studied by Becquerel), that they called Radium (1898) (Kułakowski, 2011). The two scientists initially studied the use of X-rays for diagnostic purposes but eventually realized that they were harmful at the cellular level, thus suggesting their use in the treatment of tumors (Curie and Curie, 1899). X-rays were already used in 1896 by Emil H. Grubbé for the treatment of breast cancer (Grubbé, 1933; Nakayama and Bonasso, 2016), while Anton Ultimus Sjögren applied this treatment to an epithelioma of the mouth in 1899 (Nakayama and Bonasso, 2016). However, modern radiotherapy only began in 1920, when Claudius Regaud demonstrated that radiation fractionation could be used to treat several human cancers, by reducing the side effects of the treatment itself (Deloch et al., 2016; Moulder and Seymour, 2017).

Despite these achievements, medical and interventional approaches to tumors before the Second World War were essentially radical methods, aimed at the complete eradication of the disease before it can spread and metastasize throughout the organism. Therefore, surgical treatment, often representing the only therapeutic option despite its demolition impact, resulted ineffective in patients with advanced tumor pathology or whenever the surgical act failed to remove all the tumor mass (Hajdu, 2011; Hajdu and Vadmal, 2013). An epochal turning point for the treatment of tumors was reached in the mid-1900s, with the birth of chemotherapy and the subsequent evolution of modern medical therapy of tumors. The discovery and synthesis of new compounds represented the basis to develop effective therapeutic interventions in patients with different types of advanced solid tumors or hematological drug treatments, alone or in association with surgical and radio treatments (Arruebo et al., 2011; **Figure 2**).

The first revolutionary pharmacological approach was represented by the use of chemotherapeutic antitumor drugs, which cytotoxic against various tumors; however, the toxicity to normal tissues and the development of drug resistance mechanisms by tumor cells represented important obstacles to overcome (Chabner and Roberts, 2005).

Subsequently, with the definition of the DNA structure and the development of new molecular techniques for DNA analysis, specific gene alterations responsible for neoplastic transformation were identified. These alterations were studied in order to synthesize drugs specifically targeting them, i.e., the targeted therapies, specific for certain tumors (Krause and Van Etten, 2005).

Furthermore, in the last 20 years new anti-tumor therapeutic strategies, which make use of new biotechnological drugs, have been developed. These strategies have significantly increased the effectiveness of treatments and the survival rates of cancer patients. Among these, monoclonal antibodies and new immunotherapeutic drugs have allowed the development of new personalized therapeutic protocols (personalized medicine) that have shown very high efficacy and low toxicity for patients (Scott et al., 2012; Tsimberidou, 2015). In addition, the research in the field of oncology is constantly aimed at the discovery of new and effective therapeutic strategies, including the promising CAR-T Cell therapy and gene therapy (Yescarta and Kymriah) (Gross et al., 1989; Rosenberg et al., 1990; Vile et al., 2000; Rosenbaum, 2017; Hidai and Kitano, 2018). Moreover, new therapeutic combined protocols, which use different drugs and different types of treatment, are undergoing clinical trials, in order to find therapeutic schemes that can increase the treatment efficacy and reduce the possibility of developing pharmacological resistance (Hu et al., 2010; Vanneman and Dranoff, 2012).

Finally, nowadays, the development of a new drug must necessarily include the integration of multidisciplinary skills to obtain new pharmaceutical molecules available for the market, with good safety and tolerability. In particular, the development of a new drug can no longer be exempt from an initial bioinformatics *in silico* to simulate the level of interaction of hundreds of new molecules with a specific receptor target of the new drug to be implemented. Following the bioinformatics study, it is essential to use several *in vitro* and preclinical animal models to establish the toxicity of the new drug and its therapeutic potential. Therefore, today, bioinformatics and preclinical studies are the fundamental steps to develop a new effective drug endowed with the highest potential efficacy. The *in silico* and preclinical screening of thousands of different pharmacological molecules has in fact allowed the researchers to obtain new oncological drugs which are currently used in clinical practice while significantly reducing mortality from oncological diseases.

THE BIRTH AND EVOLUTION OF CHEMOTHERAPY FOR THE TREATMENT OF TUMORS

After the discovery and application of X-rays for the diagnosis and treatment of some tumors, there has been a period of standoff for the research of new treatments to be used in cancer care. A new and significant turn to the treatment of tumors took place around the '40s of the twentieth century, during the Second World War, with the accidental discovery of the first DNA alkylating agent, a nitrogen mustard derived from iprite, used for war purposes, whose toxic effects determined bone marrow toxicity and killing of white blood cells. In particular, in December 1943, the John Harvey ship carrying nitrogen mustard bombs was bombed and the toxic gas released into the atmosphere; in the following months, almost a thousand men and women previously exposed to the gas died due to complications characterized by bone marrow aplasia (Brookes, 1990).

TABLE 1 | Milestones of oncology research before 1900.

	Historical period	Major discoveries in oncology
Ancient discoveries and theories of cancer	3000 B.C.	In Edwin Smith's papyrus the first case of human cancer is described
	1500 B.C.	Ebers' papyrus describes the tumors of the skin, uterus, stomach and rectum
	400 B.C.	Hippocrates proposes the first theory on the development of tumors
	130–200	Galen deepens the theory of Hippocrates, proposing that the excess of black bile causes incurable tumors while the excess of yellow bile causes treatable tumors
	300–400	Oribasius of Baghdad confirms that the tumors are caused by an excess of black bile
No significant progress in the study of tumors*,**	527–565	Aëtius of Amida introduces the treatment of breast tumors by amputation of the entire organ
	625–690	Paul of Aegina describes the tumors of the uterus and the surgical approach for the treatment of the bladder, the thyroid and the polypectomy of the nasal polyps
	860–932	Rhazes di Baghdad describes new treatments for tumors in the "De Chirurgia" manuscript.
	980–1037	Avicenna introduces the removal of tumors of the rectum
	1070–1162	Averroes of Cordoba describes the tumors of the esophagus and rectum and introduces the hysterectomy for the removal of uterine tumors
	1500	Paracelsus questions Hippocrates and Galen theories and hypothesizes that tumors develop due to an accumulation of "salts" in the blood
	1543	Andreas Vesalius published the manuscript "De Humani corporis fabrica" containing anatomical information resulting from post-mortem examinations
	1600	Doctors and surgeons propose that the coagulation and fermentation of blood and/or lymph are the cause of the development of tumors
	1600–1620	Invention of the microscope
	1700	Boerhaave hypothesizes that cancer is most likely induced by elements, present in water or in the ground, which defines viruses. It is theorized that chronic inflammation, injury, trauma and family predispositions can determine the development of tumors
	1760	Morgagni hypothesizes that cancer is related to pathological lesions of a particular organ
	1775	Perciaval Pott defines the association between scrotal cancer and exposure to soot in chimney sweeps
	1858	Rudolf Virchow identifies the origin of tumors in the altered cells
	1896	Wilhelm Conrad Röntgen discovers X-rays
Birth of radiotherapy	1896	Emil H. Grubbé uses X-rays to treat breast cancer
	1898	Marie and Pierre Curie discover the radiation emitted by the Radium
	1899	Marie and Pierre Curie suggest using X-rays to treat tumors
	1920	Birth of radiotherapy

**Hajdu, 2016; ***Hajdu, 2017*.

Alkylating Agents

The bone marrow toxicity of the nitrogen mustard is due to its alkylating activity toward DNA, occurring through two molecular steps; first the aziridinium group of the nitrogen mustard binds the guanine bases, then interstrand cross-links (ICLs) are formed after the displacement of a chlorine (Brookes and Lawley, 1960, 1961). The formation of ICLs is at the basis of the cytotoxic activity of nitrogen mustards, preventing DNA duplication and leading to cell death, particularly in the presence of high cell turnover. Later on, in 1946, Alfred Gilman and Louis Goodman at Yale University discovered the pharmacological effect of nitrogen mustards on organisms affected by certain tumors, such as Hodgkin's lymphoma and other lymphomas and leukemia (Gilman, 1946, 1963). Between 1946 and 1948, the first results of the clinical studies on the therapeutic efficacy of nitrogen mustards were published, formally defining the first chemotherapeutic drugs used in modern oncology (Goodman and Wintrobe, 1946; Rhoads, 1946; Faloon and Gorham, 1948).

The first nitrogen mustard to be used as an alkylating agent in clinical practice was Mechlorethamine, able to bind nitrogen N7 of guanine and to inhibit DNA replication by the above-described mechanisms. In particular, the first uses of Mechlorethamine were intended for patients with prostate cancer and in patients with lymphoid malignancies, such as Hodgkin's disease, lympho-reticulosarcomatosis and lymphatic leukemia (Kieler, 1951; Goodwin et al., 1967). First generation nitrogen mustards are no longer used, due to the high toxicity and pharmacological resistance mechanisms developed by tumor cells. Presently, the nitrogen mustard mainly used in oncological treatments is cyclophosphamide, a bischloroethylenic compound, able to interfere with the cell cycle of both active and quiescent cells (Friedman and Seligman,

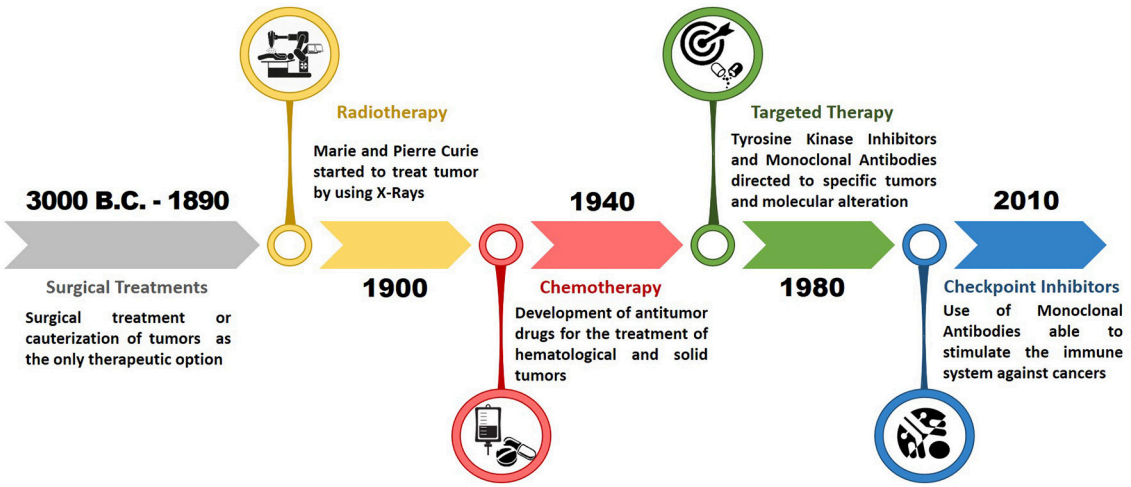

FIGURE 2 | Timeline of epochal turning points in modern oncology. After the development of radiotherapy in the early 1900, the modern oncology began with the discovery of the first chemotherapeutic drugs around 1940. Subsequently a breakthrough in the field of medical oncology occurred with the development of targeted therapy in 1980, which determined an improvement in the effectiveness of cancer treatments. The last epochal turn took place in 2010 with the introduction of immune checkpoints inhibitors for the treatment of advanced and metastatic tumors.

1954; Lane, 1959). Although cyclophosphamide can be used in the treatment of various forms of cancer, it is mostly used for the treatment of neoplastic diseases involving the immune system. It is used in the treatment of lymphoma, multiple myeloma, leukemia, ovarian cancer, breast cancer, non-small cell lung carcinoma (NSCLC), neuroblastoma, sarcoma, as well as in the treatment of several autoimmune diseases (Emadi et al., 2009; Brummaier et al., 2013; Kim and Chan, 2017).

Other alkylating agents are represented by nitrosourea compounds (carmustine, lomustine, semustin, streptozocin, nimustine, tallimustine, photemustine), alkyl sulfates (busulfan, treosulfan, mannosulfan), ethyleneimine derivatives (thiotepa, triazichinone), epoxides (etoglucide), triazene compounds (dacarbazine, temozolomide) and metal salts (cisplatin, carboplatin, oxaliplatin, satraplatin) (Puyo et al., 2014). Among these, dacarbazine and platinum compounds are alkylating agents still widely used in the first and second line treatments of various tumors. These agents are used for melanomas, Hodgkin's lymphomas, soft tissue sarcoma, NSCLC, carcinoma of the esophagus, carcinoma of the stomach, bladder cancer, genitourinary tumors, head and neck cancer, ovarian cancer, and carcinoma of the testis (Lokich, 2001; Al-Badr and Alodhaib, 2016).

In particular, dacarbazine was first synthesized by Shealy in 1962 and was approved by the Food and Drug Administration (FDA) in 1975 for the treatment of melanoma and lymphomas (Shealy et al., 1962). The discovery of platinum compounds by Rosenberg and colleagues at the Michigan State University took place in 1965, with cisplatin (first generation), and was further implemented with the synthesis of carboplatin (second generation) and oxaliplatin (third generation), which have revolutionized the treatment of several solid tumors, thanks to their broader antitumor activity and comparatively less nephrotoxicity (Rosenberg et al., 1965; Evans et al., 1983; Rossi et al., 2005).

Antimetabolites

Soon after the Second World War, new therapeutic approaches for the treatment of tumors have been developed, based on the use of molecules mimicking the structure of physiological metabolites, thereby blocking enzymatic chains essential for the synthesis of purines, which results in inhibition of cell proliferation. The main antimetabolites include folate analogs (aminopterin and methotrexate), purine analogs (mercaptopurine) and pyrimidine analogs (fluorouracil, gemcitabine, capecitabine; Kaye, 1998; Tiwari, 2012).

Antifolates were the first class of antimetabolites studied. In 1947, Sidney Farber, a pathologist at Harvard Medical School in Boston, obtained with aminopterin the first complete pharmacological remission in a child affected by acute lymphoblastic leukemia. Following this observation, aminopterin was the first folic acid analog used to reduce tumor cells proliferation and restore the bone marrow homeostasis (Farber et al., 1948; Thiersch, 1949). The remission of acute pediatric leukemia stimulated the research of other antifolate derivatives, which conserved therapeutic efficacy but exerted less toxic effects. Among the various synthesized compounds, methotrexate (amethopterin), a methylated derivative of endopterin, is still one of the most important currently available antineoplastic drugs (Meyer et al., 1950).

The mechanism of action of both aminopterin and methotrexate was not initially clear. Ten years after Faber's findings, antifolates were shown to specifically inhibit the enzyme dihydrofolate reductase (DHFR). In particular, methotrexate permanently bound DHFR, leading to inhibition of thymidylate and purine synthesis and, subsequently, to the induction of apoptosis (Jolivet et al., 1983). This mechanism of action has

proven to be very effective in limiting the tumor growth of numerous solid tumors, including breast, ovarian, head and neck, and bladder cancer (Jolivet et al., 1983). Furthermore, methotrexate has been shown to lead to complete remission of patients with choriocarcinoma and as adjuvant therapy to prevent the onset of osteosarcoma relapse after surgery (Li et al., 1958; Jaffe et al., 1974; Chabner and Roberts, 2005).

In the early '50s, other antimetabolites were synthesized and many of them are still used nowadays. Among these, 6-mercaptopurine and 5-fluorouracil, analogs of purines and pyrimidines, respectively, are widely used in clinical practices for the treatment of both hematological malignances and solid tumors (De Abreu et al., 2000; Wei et al., 2018). In 1954, Skipper and Hitchings studied the purine analogs by developing a drug, 6-mercaptopurine, able to compete with hypoxanthine and guanine for the synthesis of their nucleotide derivatives (Hitchings and Elion, 1954; Skipper et al., 1954). Furthermore, following its conversion into thioinosinic acid (TIMP), 6-mercaptopurine in turn inhibits several enzymatic reactions, including the formation of 5′-adenylic acid (AMP) fundamental for DNA and RNA synthesis. 6-mercaptopurine treatment has been shown to be particularly effective in patients with acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML).

Finally, the pyrimidine analogs made their entry in cancer clinical practice with the introduction of 5-fluorouracil (5-FU). In 1957, Charles Heidelberger synthesized 5-FU, which has revolutionized the treatment of gastrointestinal tumors with particular reference to colorectal cancer, where 5-FU is still actively used in association with others anticancer drugs in several protocols, such as the FOLFOX and FOLFIRI regimens (Heidelberger et al., 1957; Carrillo et al., 2015).

Antimitotics of Natural Origin

The important medical and pharmacological findings obtained in the early '40s and '50s have revolutionized the therapeutic approaches for cancer, leading to significant improvements in survival rate, especially for patients with onco-hematological diseases (Frei, 1985). A new boost to the chemotherapeutic treatment of both hematological and solid tumors has occurred with the introduction in the therapeutic practice of natural extracts with cytotoxic activity, able to interfere with the formation of microtubules and to block the mitotic processes and cell proliferation (van Vuuren et al., 2015). These compounds were commonly classified as microtubule-destabilizing agents or microtubule-stabilizing agents (Chen and Horwitz, 2002) because they act either by inhibiting the polymerization of microtubules via the interaction with the spindle assembly checkpoint (SAC) or by stabilizing microtubules and prevent Ca^{2+}- or cold-induced depolymerization, with subsequent blockage of mitotic fuse disassembly (Chen and Horwitz, 2002).

The first antimitotic agents discovered in the late '50s were extracted from the plant *Catharanthus roseus* (rosy periwinkle), and took the name of Vinca alkaloids. Vinca alkaloids were firstly used for the treatment of diabetes, but further studies by Noble and colleagues showed the carcinostatic activity against transplantable mammary adenocarcinoma and sarcoma in mouse models (Noble et al., 1958). Subsequently, in 1963, Johnson and colleagues elucidated the molecular mechanisms underlying the effect of Vinca on tumor cell proliferation (Johnson et al., 1963). In the following years, numerous Vinca derivatives were synthesized, all with depolymerizing action against microtubules. Among these molecules, those with greater therapeutic efficacy were vinblastine, vincristine, vinorelbine, vindesine, etc. (Jordan and Wilson, 2004). All these drugs are still widely used in first and second line therapy for the treatment of various forms of cancer (acute lymphocytic leukemia, malignant lymphomas, multiple myeloma, metastatic breast cancer, small cell lung carcinoma (SCLC), Ewing's sarcoma, embryonal rhabdomyosarcoma, etc.) representing a relevant pharmacological option for patients developing drug resistance to other chemotherapeutic agents. Finally, as described at the end of this chapter, Vinca alkaloids are used in several combined therapeutic regimens.

Other natural antimitotic agents are derived from *Podophyllum peltatum* (mayapple, wild mandrake) from which podophyllin is obtained, initially used for the treatment of epitheliomas and sarcomas with a high toxicity. All *Podophyllum* derivatives, called epipodophyllotoxins, determine the arrest of cell proliferation by blocking the topoisomerase II, fundamental for DNA unwinding during the duplication phase (Imbert, 1998). Given its generalized toxicity, derivatives with higher selectivity and fewer side effects were obtained, including the recently revoked teniposide (VM-26) and etoposide (VP-16) still used in polychemotherapy schedules for the treatment of SCLC, acute monoblastic leukemia and non-seminomatous testis carcinomas (Minocha and Long, 1984).

Finally, the class of antimitotic agents includes topoisomerase I inhibitors (topotecan, irinotecan) and microtubule stabilizing molecules, of which taxanes (paclitaxel, docetaxel, cabazitaxel) represent the most important compounds (Oberlies and Kroll, 2004). In particular, irinotecan is the latest derivative of camptothecin, extracted from an ornamental Chinese tree, *Camptotheca acuminate* (happy tree, cancer tree, or tree of life), in 1966 by Wall and Wani at the Research Triangle Institute (Wall et al., 1966). Irinotecan has been shown to have much more effective antitumor activity than first-generation camptothecins and less renal toxicity. Since 1996, irinotecan has been approved for the treatment of colorectal carcinoma, alone or in combination with 5-fluorouracil or platinum compounds, and subsequently also used for the treatment of NSCLC and ovarian cancer (Rothenberg, 1996; Rosen, 1998).

Five years later, in 1971, the same research group of Wall and Wani described the molecular structure of taxol, a natural compound with antimitotic properties extracted from the tree *Taxus brevifolia* (Pacific yew or western yew, Wani et al., 1971). However, only in 1979, Susan B. Horwitz and her research group described the mechanism of action of Taxol, highlighting its activity as microtubule stabilizer (Schiff et al., 1979). Several drugs have been derived from the Taxol. The progenitor is represented by paclitaxel, still used in clinical practice. Later on, second- (docetaxel) and third-generation (cabazitaxel) derivatives were developed (Bissery et al., 1991; Mita et al., 2009). All these compounds have revolutionized the treatment of several solid tumors including metastatic breast

cancer, metastatic pancreatic adenocarcinoma (in association with gemcitabine), NSCLC (in association with carboplatin), head and neck cancer, gastric and prostatic cancer. In particular, these drugs are used when the first line treatment failed in metastatic patients and therefore represent the only therapeutic option for patients who show drug resistance mechanisms or are not candidates for curative surgical interventions (Ojima et al., 2016).

Cytotoxic Antibiotics and Related Substances

Among the standard chemotherapeutic drugs, there are also some antibiotics and/or their derivatives with marked cytotoxic activity, which are among the most effective anticancer drugs currently used in different therapeutic regimens (Weiss, 1992). A wide range of natural antibiotics displays cytotoxic effects; their main mechanism of action is to form covalent bonds with nucleic acids, interfering with DNA synthesis. The first anti-tumor antibiotic used was puromycin. This is an analog of the adenine capable of integrating within the tRNA molecules on ribosomes and blocking protein synthesis by premature termination of the amino acid chain. However, puromycin was not widely used due to its non-selectivity and high systemic toxicity (Wright et al., 1955).

The discovery of the antitumor properties of the currently used antibiotics in cancer is the result of the active collaboration between European pharmaceutical companies and renowned International cancer research centers (Cassinelli, 2016).

The discovery of anthracyclines is the result of the scientific agreement between the Farmitalia and the Istituto Nazionale dei Tumori in Milan directed by Bucalossi. For the first time, a research center and a company worked together for the discovery and development of a new drug with anticancer properties. For this purpose, in 1960 the constituted workgroup started to study a Streptomyces strain, *Streptomyces peucetius,* found near Castel del Monte (Apulia). A new natural antitumor drug, called daunomycin (in a second moment called daunorubicin), was obtained from this Streptomyces strain and showed higher efficacy compared to others antitumor drugs in patients with chronic lymphoproliferative diseases (Di Marco et al., 1963; Bonadonna et al., 1968). Subsequently, in 1968, a new molecule was extracted from a mutated strain of *Streptomyces peucetius*, obtained by treating the microorganism with N-Nitroso-N-methylurea. This new antitumor drug, named adriamycin, was eventually renamed doxorubicin. The discovery of doxorubicin is the result of the collaborative effort of Farmitalia, the researchers of the Istituto Nazionale Tumori and of researchers at the Memorial Sloan-Kettering Cancer Center in New York. Doxorubicin showed better activity against tumors in mouse and a greater therapeutic index, however, the cardiotoxicity typical of anthracycline was not eliminated (Arcamone et al., 1969; Di Marco et al., 1969). The mechanism of action of anthracyclines consists in the inhibition of DNA and RNA synthesis by interfering with the topoisomerase II enzyme, preventing the relaxing of supercoiled DNA and thus blocking DNA transcription and replication (Hortobágyi, 1997).

Around the mid-'50s, the anti-tumor antibiotic actinomycin D was obtained from another strain of Streptomyces, *Streptomyces griseus*. This drug was used for the treatment of some sarcomas, choriocarcinoma, and lymphomas. Another anti-tumor drug is the mithramycin belonging to the group of DNA intercalates with high specificity for bone tumors and bone metastases (Kersten et al., 1966).

Finally, another antitumor antibiotic, still widely used in clinical practice today, is bleomycin, discovered in Japan in 1966 and immediately approved for the treatment of various tumors, such as Hodgkin's and NHL, testicular cancer, and cervical cancer, among others (Umezawa et al., 1966; Chen and Stubbe, 2005; Bolzán and Bianchi, 2018).

Other Anti-cancer Drugs: Polyamine Inhibitors and Iron-Modulating Drugs

Cell proliferation and tumor growth are promoted by several stimulating factors including polyamines, organic compounds bearing two or more amino groups, responsible for cell growth, gene transcription, translation, and chromatin remodeling (Miller-Fleming et al., 2015). On these bases, new synthetic drugs were developed in order to prevent the formation of polyamines by inhibiting their synthesis or prevent their transport across the cell membrane. The first polyamine inhibitor, an ornithine decarboxylase inhibitor, was synthesized in the 1970s and was used in clinical practices in 1980 for the treatment of trypanosomiasis and other parasitic infections (Abdel-Monem et al., 1974; Bacchi et al., 1980). Among the ornithine decarboxylase inhibitors, α-difluoromethylornithine (DFMO) is the most widely used both for parasitic infections, excess of facial hair in women, and chemotherapy. DFMO was firstly used in cancer therapy in 1981 for the treatment of kidney and bladder cancer in order to induce the reduction of tumor growth (Dunzendorfer, 1981). Subsequently, numerous studies showed the clinical efficacy of the treatment with DFMO in several cancer types (Gerner and Meyskens, 2004; Damiani and Wallace, 2018). Despite the success of DFMO, DFMO treated cells often up-regulate polyamine transport activity making the treatment ineffective. For this purpose, other molecules, called polyamine transport inhibitors (PTIs), have been produced against polyamine transporters at the cell membrane level. Today these inhibitors are generally used in combination with the DFMO showing that combination therapy is more effective in reducing intracellular polyamine levels, thereby limiting tumor growth (Muth et al., 2014).

Other anti-cancer drugs were developed to regulate the intracellular levels of iron, whose alteration may lead to cancer development. Notably, iron is an essential micronutrient for cellular homeostasis. Iron deficiency is often associated with anemia, while iron increased levels induce oxidative stress to tissues, leading to inflammation, system dysfunction that may lead to genetic alteration and consequently neoplastic transformation. Accordingly, numerous studies tried to develop new iron-modulating agents for the treatment of cancer patients. One of the used iron-modulating anti-cancer drugs is desferrioxamine (DFX), an iron chelator that reduces iron

levels and consequently its metabolism, which affects the methylation levels in CRC model (Cao et al., 2018). Among iron chelators, there are also di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone (Dp44mT), ciclopirox, and triapine, for which several clinical trials are undergoing (Fischer-Fodor et al., 2015). In particular, triapine is one of the most used drugs in clinical oncology for the treatment of several solid tumors, including uterine cervix and vaginal cancers, prostate cancer, pancreatic cancer, and advanced/metastatic solid tumors (Fischer-Fodor et al., 2015).

Combination Chemotherapy Regimens

During the '60s and the '70s, new combined therapeutic protocols using several chemotherapeutic drugs with different mechanisms of action began to be proposed in clinical practice.

The use of combined chemotherapy or anticancer polychemotherapy has represented an epochal turning point for the treatment of tumors, because achieves greater therapeutic efficacy than the use of single chemotherapeutic agents. In particular, combination therapy kills a larger number of tumor cells with a higher dose of each single drug, therefore not exceeding the maximum tolerated doses of each single drug. Furthermore, it guarantees a wider range of interaction between drugs and cancer cells with different genetic abnormalities. Finally, it is able to prevent or slow down the subsequent development of drug resistance (Lilenbaum et al., 2005).

In 1964, for the first time, Vincent De Vita and his collaborators, at the National Cancer Institute in Bethesda, proposed a combined approach for the treatment of Hodgkin's lymphoma. This first combination was named MOPP, from the initials of the four antitumor agents used: Mustargen (mechlorethamine), Oncovin (also known as Vincristine); Procarbazine and Prednisone (Moxley et al., 1967). The use of MOPP regimen achieved important therapeutic results, with complete remission in 80% of patients and no signs and symptoms of disease in the following 5 years for 60% of patients (De Vita et al., 1970). This study represented a milestone in the treatment not only for malignant lymphomas, but also for other cancers.

In the following years, thanks to studies carried out in animal models, it was shown that chemotherapeutic drugs were more effective against small tumors and when used in combination, thus establishing the importance of early diagnosis and early treatment of tumors in both adjuvant and neoadjuvant regimens. Based on these studies, new combined treatments were proposed. In June 1972, Gianni Bonadonna and Umberto Veronesi proposed a study to evaluate the efficacy of an adjuvant chemotherapy after surgery, based on the combination of three drugs Cyclophosphamide, Methotrexate, and Fluorouracil, named CMF, which improved the probability of survival of cancer patients (De Lena et al., 1973). In 1973, Bonadonna and some of his collaborators proposed a new combination of four drugs, Adriamycin, Bleomycin, Vinblastine, and Dacarbazine, named ABVD after their initials, for the treatment of Hodgkin's lymphoma. The results demonstrated that, even years later, the ABVD combination healed more patients than MOPP alone and was better tolerated, with minor side effects (Bonadonna et al., 1975).

These first studies on the efficacy of combination therapy have paved the way for the development of numerous therapeutic regimens, still used today, that have proven to be more effective than treatment with individual antitumor drugs. In the following years, the clinical importance of the relationship between the dose intensity and therapeutic efficacy of the administered drugs was confirmed and the first therapeutic protocols based on bone marrow transplantation in leukemic patients were developed.

The discoveries in the field of molecular biology gave a major impulse to develop new targeted therapies and new selective biological drugs, specific for certain tumors. These discoveries prompted the second pharmacological revolution in cancer therapy, that began in '80s, with the development of selective kinase inhibitors and monoclonal antibodies.

THE REVOLUTION OF TARGETED THERAPY: SELECTIVE KINASE INHIBITORS AND MONOCLONAL ANTIBODIES

At the beginning of the '80s, the new discoveries in the field of immunology, cell biology, and molecular biology allowed the researchers to investigate the molecular mechanisms responsible for the neoplastic transformation of cells and thus identify new molecular targets to be blocked by small selective inhibitory molecules or monoclonal antibodies. In particular, unlike the classic chemotherapy approach, which acts on both normal cells and cancer cells, the targeted therapy intervenes on altered key oncogenes or tumor suppressor genes involved in tumor promotion. By this way, these new selective inhibitors are able to affect only altered cancer cells with minor side effects toward the normal cells (Hartmann et al., 2009).

Nowadays, the term "targeted therapy" refers to all those treatments affecting specific molecular targets; this approach takes advantage of either small molecules obtained by chemical synthesis or biological drugs (also called biotechnological drugs), i.e., recombinant proteins, mainly monoclonal antibodies, directed toward specific cellular receptors and proteins involved in neoplastic processes (Tsimberidou, 2015; **Figure 3**).

The discoveries of 1975 by George Köhler and César Milstein have opened the way for the production of several specific hybrid monoclonal antibodies specific to different antigens or cellular targets, obtained by the realization of hybridomas, resulting from the fusion of murine B lymphocytes and human myeloma cells, capable of producing large quantities of monoclonal antibodies (Köhler and Milstein, 1975).

The first targeted approach to the treatment of cancer dates back to the early '80s, with the development of a monoclonal antibody tested on murine models (Bernstein et al., 1980). In 1980, Nadler and colleagues treated a patient affected by NHL with the murine monoclonal antibody AB89, but the treatment did not induce a significant clinical response (Nadler et al., 1980). Nevertheless, this was the first attempt of targeted therapy by using a monoclonal antibody able to selectively target tumor cells

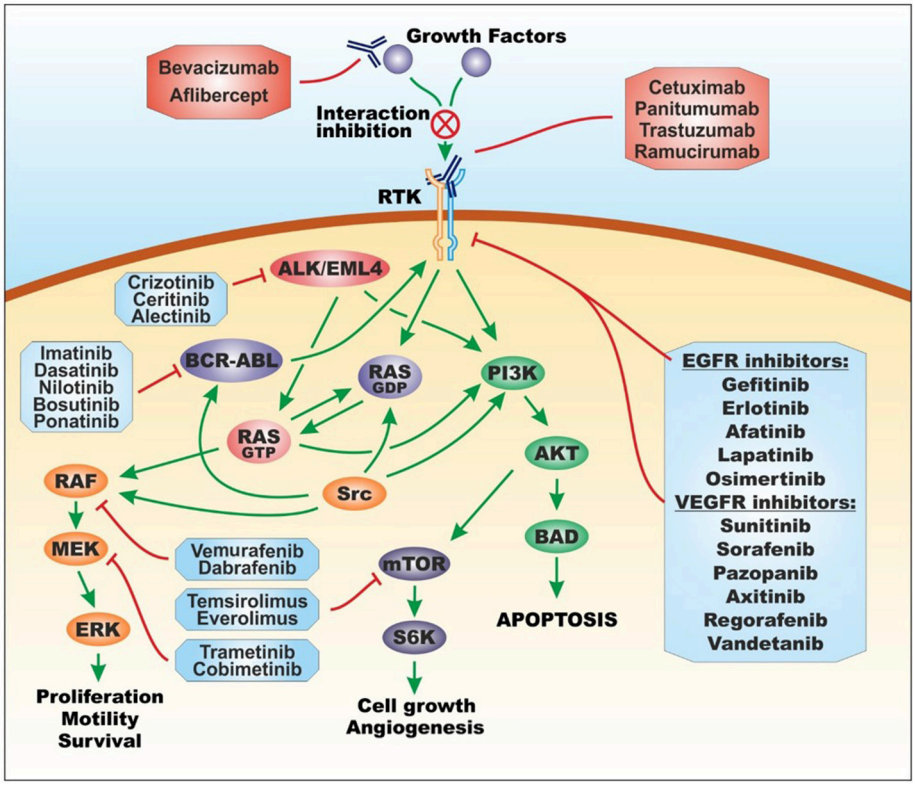

FIGURE 3 | Molecular targets of targeted therapy. Targeted therapy for cancer treatment is based on tyrosine and serine/threonine protein kinase inhibitors and monoclonal antibodies. Protein kinase inhibitors are divided into EGFR inhibitors, VEGFR inhibitors, BCR/ABL inhibitors, ALK/EML4 inhibitors, RAF inhibitors, MEK inhibitors, and mTOR inhibitors. Monoclonal antibodies are directed toward extracellular growth factors or extracellular receptor tyrosine kinase. **Figure 3** has been adapted and enriched by taking a cue from two published papers by Massimo Libra, co-author of the present review (Russo et al., 2014; Leonardi et al., 2018). For the general structure of **Figure 3** and the name of drugs, the information contained in the book "Farmacologia: Principi di base e applicazioni terapeutiche" was taken into account (Rossi et al., 2016). ABL, Abelson murine leukemia viral oncogene homolog; AKT, protein kinase B; ALK, anaplastic lymphoma kinase; BAD, Bcl-2-associated death promoter; BCR, breakpoint cluster region; EGFR, epidermal growth factor receptor; EML4, echinoderm microtubule-associated protein-like 4; ERK, extracellular signal–regulated kinases; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; RAF, rapidly accelerated fibrosarcoma kinase; RAS, RAS proto-oncogene GTPase; RTK, receptor tyrosine kinase; S6K, S6 kinase; src, proto-oncogene tyrosine-protein kinase Src; VEGFR, vascular endothelial growth factor receptor.

and to induce cell death by direct or indirect mechanisms. These two mechanisms of action are respectively defined as the direct inhibition of a molecular pathway involved in tumor progression or the enhancement of host defense mechanisms through activation of the antibody-dependent cytotoxic pathway and complement-mediated cytotoxicity (Oldham, 1983; O'Mahony and Bishop, 2006).

Other attempts to develop effective monoclonal antibodies against myelo- and linfo-proliferative diseases and lymphomas have taken place over the '80s, but without results convincing enough to justify their use in clinical practice (Miller et al., 1982). In particular, since most antibodies were murine, they induced host immune reactions against the administered drug, with subsequent side effects (allergic/anaphylactic reactions) and enhanced clearance/reduced half-life (Dillman et al., 1986; Kornbrot et al., 1994).

It was only in the '90s that the first really effective targeted therapy drugs became available. The breakthrough was made possible thanks to the studies on the human genome, and the advancement in technologies for DNA sequencing, genomics, transcriptomics and proteomics, invaluable for recognizing new molecular targets (Tsimberidou, 2015). Moreover, in those years, thanks to the new knowledge in molecular and cellular fields and the advancement of the technologies for drug discovery, we have witnessed the birth of modern targeted therapy and personalized medicine. As described below, the possibility of having effective and specific drugs against growth factors and their receptors, cytoplasmic proteins, and signal transducers altered in specific tumor pathologies lead to significant improvement in therapeutic efficacy and survival rates of cancer patients.

Monoclonal Antibodies in Cancer Therapy

Since the discovery of Trastuzumab (Herceptin®) and the first clinical trials performed in 1992, several monoclonal antibodies were discovered and introduced in cancer clinical practice. As previously mentioned, Köhler and Milstein (1975) revolutionized anti-cancer therapeutics with the development of hybridoma technology, used to produce monoclonal antibodies. Initially,

the produced monoclonal antibodies were mouse antibodies. Subsequently, thanks to the new genetic engineering techniques, it was possible to obtain different types of monoclonal antibodies used for the treatment of both hematological and solid tumors. In particular, there are 4 types of monoclonal antibodies available: murine, chimeric, humanized and human monoclonal antibodies, which differ each other by the percentage of murine protein portion present in the immunoglobulin (Pento, 2017).

According to the last report of the "Animal Cell Technology Industrial Platform" (ACTIP), in 2017, 30 different monoclonal antibodies were approved by FDA and/or EMA for the treatment of hematological and solid tumors and others will be approved in the near future; others 6 antibodies were approved for the diagnosis of cancers (**Table 2**; ACTIP, 2017).

Both EMA and FDA have released various regulatory guidelines concerning development, clinical experimentation, approval and subsequent commercialization of monoclonal antibodies used in anti-tumor therapies. In particular, in September 2017 EMA released the fifth version of the "Guideline on the evaluation of anticancer medicinal products in man" providing the guidance for the development of anti-cancer drugs, including monoclonal antibodies, in all stages of their clinical development (European Medicine Agency, 2016, 2017). According to EMA, the development of a monoclonal antibody is a multi-step process composed by *in vitro* non-clinical studies performed to elucidate the prime activity of the drug and subsequent pre-clinical studies in animal models and clinical trials in tumor patients to assess the pharmacokinetics, clearance, activity and response to monoclonal antibody treatments (European Medicine Agency, 2016, 2017). Regarding FDA regulations, a document containing all the guidance for the production and regulation of monoclonal antibody drugs was published in 1997, "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (Food Drug Administration, 1997). Subsequently, several drafts were approved and published by FDA, but the 1997 document still remains the the main reference text for development and production of new monoclonal antibodies used in oncology and for the treatment of other diseases.

As mentioned above, Trastuzumab was the first monoclonal antibody tested in a clinical trial (1992) directed toward the mutated HER2/neu receptor of breast cancer (Carter et al., 1992). However, the approval of this drug by the regulatory agencies took place only in 1998 (Miller, 1998). Because of this delay, the first approved monoclonal antibody was Rituximab (Rituxan® and MabThera®), approved in 1997, a year earlier than Trastuzumab. The introduction of both Rituximab and Trastuzumab have represented landmark events in the revolution of anti-tumor treatments.

In particular, the first experiments on Rituximab were carried out in 1994 by IDEC Pharmaceuticals Corporation, which, inspired by the studies conducted on murine monoclonal antibodies against the surface antigen CD-20, developed a chimeric monoclonal antibody, named IDEC-C2B8, able to determine the killing of B-cells in both monkeys and B-cell lymphoma patients (Maloney et al., 1994; Reff et al., 1994). Subsequently, in 1997, FDA gave the final approval for the use of this antibody also in patients affected by lymphoproliferative B lymphocyte disorders.

After the approval, all the phase II and III clinical trials showed the promising effects of Rituximab in the treatment of several types of refractory NHLs, including mantle cell and diffuse large B-cell lymphomas (Coiffier et al., 1998). Others studies showed that high doses of Rituximab were well-tolerated by patients affected by indolent NHL and were able to induce the remission of pathology in a high percentage of patients (Ghielmini et al., 2004; Hainsworth et al., 2005). Furthermore, in 2002, a combination therapy including Rituximab and CHOP standard chemotherapy (Cyclophosphamide, Hydroxydaunorubicin (also called doxorubicin), Oncovin (vincristine), and Prednisone or Prednisolone) was proposed (Coiffier et al., 2002; Mounier et al., 2003). The so-called R-CHOP regimen has shown a significant outcome improvement for patients affected by NHLs. Another antibody directed to the CD-20 receptor is Tositumomab, a murine IgG2a lambda monoclonal antibody, produced in mammalian cells. This monoclonal antibody was eventually conjugated with iodine 131 (Iodine I 131 Tositumomab). Both labeled and unlabeled Tositumomab are approved for the treatment of Rituximab-refractory NHLs (Quackenbush et al., 2015).

Regarding Trastuzumab, it is a humanized monoclonal antibody obtained by genetic engineering technologies, able to inhibit the activation of human epidermal growth factor receptor 2 (HER2)/neu, a glycoprotein receptor with tyrosine kinase activity, which, when altered, promotes breast cancer cells growth. Beside receptor blockade, Trastuzumab is able to induce cancer cell death by Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC), a key mechanism also exploited by other monoclonal antibodies (Hudes et al., 2007). Trastuzumab was obtained by Ullrich and Shepard at UCLA's Jonsson Comprehensive Cancer Center (Shepard et al., 1991). Subsequent collaboration between Genentech and UCLA scaled up the development of Trastuzumab that was used in the first clinical trial in 1992 (Carter et al., 1992).

The results obtained by the first phase I and II trials suggest that Trastuzumab, through the repression of the HER2/neu receptor, reduced the aggressiveness of breast cancer cells. Furthermore, these studies showed that Trastuzumab was effective when used in monotherapy as well as in combination with platinum compounds (Baselga et al., 1996; Pegram et al., 1998). The effectiveness and safety of Trastuzumab have made it the gold standard treatment for women with metastatic breast cancer, alone or in combination with paclitaxel or doxorubicin, and for patients affected by HER2-positive metastatic gastric carcinoma (Müller et al., 2018). Finally, in 2013 a conjugated monoclonal antibody was approved, named Trastuzumab-emtansine. This is an antibody-drug conjugate composed by the monoclonal antibody Herceptin chemically linked with the antimitotic agent emtansine (DM1 or mertansine; Niculescu-Duvaz, 2010; LoRusso et al., 2011). This drug showed a higher efficacy compared to Trastuzumab alone because while the monoclonal antibody inhibits the cell growth through its interaction with the HER2/neu receptor and subsequent inhibition of both MAPK and PI3K/AKT cellular signaling

TABLE 2 | Monoclonal antibodies approved by the EMA and FDA for cancer treatment and diagnosis –2017 Update.

Trade name	Active principle name	Company	Target	Type	Year of EU EMA approval	Year of FDA approval	Therapeutic indication(s)
Bavencio®	Avelumab	Merck Sharp & Dohme Limited	PD-L1	Human IgG1/κ	Not approved*	2017	Metastatic Merkel cell carcinoma
Imfinzi®	Durvalumab	Astrazeneca UK	PD-L1	Human IgG1/κ	Not approved*	2017	Metastatic urothelial carcinoma
Lartruvo	Olaratumab	Eli Lilly	PDGFR-α	Human IgG1	2016	2016	Sarcoma
Darzalex®	Daratumumab	Janssen-Cilag	CD38	Human IgG1/κ	2016	2015	Multiple myeloma
Empliciti	Elotuzumab	Bristol-Myers Squibb	SLAMF7	Human IgG1	2016	2015	Multiple myeloma
Portrazza	Necitumumab	Eli Lilly	EGFR	Human IgG1	2016	2015	Carcinoma, non-small-cell lung
Tecentriq®	Atezolizumab	Genentech (Roche)	PD-L1	Human IgG1	Not approved*	2016	Metastatic non-small cell lung cancer
Opdivo	Nivolumab	Bristol-Myers Squibb Pharma	PD-1	Human IgG4	2015	2015	Carcinoma; non-small-cell lung carcinoma; renal cell Hodgkin disease melanoma
Unituxin	Dinutuximab	United Therapeutics Europe	GD2	Human IgG1/κ	2015 (1)	2015	Neuroblastoma
Blincyto®	Bevacizumab	Amgen Europe	CD19	BiTEs	2015	2014	Precursor cell lymphoblastic leukemia-lymphoma
Keytruda®	Pembrolizumab	Merck Sharp & Dohme Limited	PD-1	Human IgG4	2015	2014	Melanoma
Cyramza	Ramucirumab	Eli Lilly	VEGF	Human IgG1	2014	2014	Stomach neoplasms
Perjeta®	Pertuzumab	Roche	HER2	Humanized IgG1	2013	2012	Breast cancer
Gazyvaro®	Obinutuzumab	Roche	CD20	Humanized IgG1	Not approved*	2013	CLL
Vervoy®	Ipilimumab	BMS	CTLA-4	Human IgG1	2011	2011	Melanoma
Xgeva®	Denosumab	Amgen	RANKL	Human IgG2	2011	2011	Prevention of SREs in patients with bone metastases from solid tumors
Arzerra®	Ofatumumab	Genmab and GSK	CD20	Human IgG1	2010	2009	Chronic lymphocytic leukemia
Removab®	Catumaxomab	Fresenius	EpCAM and CD3	Trifunctional MAb IgG2a/IgG2b	2009	Not approved*	Malignant ascites in patients with EpCAM-positive carcinomas
Vectibix®	Panitumumab	Amgen	EGFR	Human IgG2	2007	2006	Metastatic colorectal carcinoma
Proxinium®	Catumaxomab	Viventia (Eleven Biotherapeutics)	EpCAM	Humanized MAb	2005	2005	Head and neck cancer
Avastin®	Bevacizumab	Genentech (Roche)	VEGF	Humanized IgG1	2005	2004	Metastatic colorectal cancer; non-small cell lung cancer; metastatic breast cancer; glioblastoma multiforme; metastatic renal cell carcinoma
Erbitux®	Cetuximab	ImClone (Eli Lilly), Merck Serono and BMS	EGFR	Chimeric IgG1	2004	2004	Head and neck cancer; colorectal cancer
Campath®	Alemtuzumab	Millennium Pharmaceuticals and Genzyme	CD52	Humanized IgG1	2001	2001	B-cell chronic lymphocytic leukemia

(Continued)

TABLE 2 | Continued

Trade name	Active principle name	Company	Target	Type	Year of EU EMA approval	Year of FDA approval	Therapeutic indication(s)
Herceptin®	Trastuzumab	Genentech (Roche)	HER-2	Humanized IgG1	2000	1998	Breast cancer; metastatic gastric or gastroesophageal junction adenocarcinoma
Rituxan® MabThera®	Rituximab	Biogen Idec, Genentech (Roche)	CD20	Chimeric IgG1	1998	1997	Non-Hodgkin's lymphoma; chronic lymphocytic leukemia; rheumatoid arthritis
CONJUGATED MONOCLONAL ANTIBODIES FOR CANCER TREATMENT							
Zevalin®	Ibritumomab tiuxetan	Biogen Idec	CD20	Murine IgG1	2004	2002	Non-Hodgkin's lymphoma
Bexxar®	Tositumomab and iodine 131 tositumomab	Corixa and GSK	CD20	Murine IgG2a	Not approved*	2003	Non-Hodgkin's lymphoma
Mylotarg®	Gemtuzumab ozogamicin	Wyeth	CD33	Humanized IgG4/toxin conjugate	Not approved*	2000 (2)	Acute myeloid leukemia (AML)
Kadcyla®	Trastuzumab emtansine	Roche	HER2	Humanized IgG1 as ADC	2013	2013	Breast cancer
Adcetris®	Brentuximab	Seattle Genetics	CD30 + MMAE	Chimeric IgG1 as ADC (antibody drug conjugate)	2012	2011	Hodgkin lymphoma (HL), systemic anaplastic large cell lymphoma (ALCL)
DIAGNOSTIC MONOCLONAL ANTIBODIES FOR CANCER							
Humaspect®	Votumumab	Organon Teknica	Cytokeratin tumor-associated antigen	Human Mab + 99mTc	1998 (3)	Not approved*	Detection of carcinoma of the colon or rectum
LeukoScan®	Sulesomab	Immunomedics	NCA90	Murine Fab fragment	1997	Not approved*	Diagnostic imaging for osteomyelitis
CEA-scan®	Arcitumomab	Immunomedics	Human CEA	Murine Fab fragment	1996 (4)	1996	Detection of colorectal cancer
ProstaScint®	Capromab	Cytogen	Tumor surface antigen PSMA	Murine MAb	Not approved*	1996	Detection of prostate adenocarcinoma
Verluma®	Nofetumomab	Boehringer Ingelheim, NeoRx	Carcinoma-associated antigen	Murine Fab fragment	Not approved*	1996	Diagnostic imaging of small-cell lung cancer
OncoScint®	Satumomab	Cytogen	TAG-72	Murine MAb	Not approved*	1992	Detection of colorectal and ovarian cancers

Not yet approved by EMA or FDA; (1). Withdrawn from use in the European Union; (2). Withdrawn from the market in US in 2010; (3). Withdrawn from the market in EU in 2003; (4). Withdrawn from the market in EU in 2005.

pathways, emtansine entries the cell and binds to tubulin, preventing the duplication of DNA (Barok et al., 2014). The first clinical trials showed that Trastuzumab-emtansine significantly improves patient's progression-free survival (PFS) (14.2 months, compared to 9.2 months for patients treated with the standard regimen Trastuzumab plus docetaxel; Hurvitz et al., 2013).

The HER2/neu receptor is also the target of another humanized monoclonal antibody, Pertuzumab (Perjeta®), used in patients affected by HER2-positive metastatic and non-metastatic breast cancer. Pertuzumab is often administered in association with Trastuzumab and docetaxel in adjuvant and neoadjuvant regimens (Schneeweiss et al., 2013). Pertuzumab was discovered and developed by Genentech and then approved in 2012. Similar to Trastuzumab, Pertuzumab is a HER dimerization inhibitor; in particular it prevents the dimerization of HER2 with other HER receptors thus inhibiting intracellular phosphorylation events, which subsequently blocks the abnormal cell growth and proliferation (Harbeck et al., 2013).

The third monoclonal antibody directed to surface receptors discovered in order of time is Cetuximab (Erbitux®) directed against the epidermal growth factor receptor (EGFR), frequently altered in numerous tumors, especially colorectal carcinomas, NSCLC and head and neck cancers (Vokes and Chu, 2006). Cetuximab is a chimeric (mouse/human) monoclonal antibody with a 5- to 10-fold higher affinity for EGFR compared to the endogenous ligands. Its mechanism of action consists in the inhibition of EGFR signaling transduction pathway resulting in the block of cell cycle progression, angiogenesis, cell migration and invasion; furthermore, Cetuximab is able to promote ADCC cytotoxicity and to induce EGFR internalization, resulting in down-regulation of EGFR itself (Vincenzi et al., 2010). Since 1988, Sela and collaborators have studied a monoclonal antibody directed to EGFR and observed a significant therapeutic effect in cellular and pre-clinical models of human carcinomas (Aboud-Pirak et al., 1988). Later on, thanks again to the support of a pharmaceutical company, Cetuximab entered clinical development and showed immediately important results in of phase II and III clinical trials carried out on patients affected by colorectal carcinomas, NSCLC and head and neck cancers (Robert et al., 2001; Kim, 2004; Saltz et al., 2004). This treatment, however, is effective only in the subset of patients without activating mutation of KRAS gene (Lièvre et al., 2006).

In the following years, another human monoclonal antibody, Panitumumab (Vectibix®), directed to EGFR was developed. In 2006, Panitumumab was approved for the treatment of metastatic colorectal cancer patients with wild-type KRAS and refractory to standard chemotherapeutic regimens (Poulin-Costello et al., 2013).

The monoclonal antibodies described above, with the exception of Rituximab directed against the CD-20 differentiation cluster, are all directed to extracellular receptors responsible for the activation of various molecular pathways that have, as their final effect, the increase in cell proliferation and/or inhibition of apoptosis (Fauvel and Yasri, 2014). Another monoclonal antibody widely used for the treatment of different types of solid tumors is Bevacizumab (Avastin®) a recombinant humanized monoclonal antibody directed to a soluble growth factor and not to a receptor. Indeed, Bevacizumab blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A) representing the first anti-angiogenic factor to be developed (Chen et al., 2001).

The discovery and development of Bevacizumab began with studies by Senger in 1983, who for the first time identified the vascular endothelial growth factor responsible for neovascularization observed in rodents' tumors (Senger et al., 1983). Subsequently, in 1989 the research group directed by Napoleone Ferrara purified and cloned VEGF (Ferrara and Henzel, 1989), starting the development of Bevacizumab (Presta et al., 1997).

The discovery of Bevacizumab and its use in clinical practice represented an epochal turning point for the first and second line treatment of numerous metastatic solid tumors, for which no effective treatments were yet available. The first clinical trials (1997) showed that bevacizumab, used as a single agent, was well-tolerated by patients and when administered in combination with other chemotherapeutic agents did not lead to an increase in systemic toxicity (Gordon et al., 2001). Further phase II and III clinical studies demonstrated the efficacy of Bevacizumab, administered alone, for patients with renal cell carcinoma, or, in combination with standard chemotherapy, for patients with colorectal carcinoma and NSCLC (Ferrara et al., 2004).

Today Bevacizumab is used for the treatment of metastatic colorectal carcinoma, advanced or metastatic breast cancer, advanced metastatic lung cancer, advanced and/or metastatic renal carcinoma, epithelial ovarian carcinoma, fallopian tubes carcinoma, peritoneal carcinoma and recurrent or metastatic cervix carcinoma (Keating, 2014).

Other antibodies used for the treatment of tumors are: Brentuximab (Adcetris®), Ofatumumab (Arzerra®), Alemtuzumab (Campath®), Obinutuzumab (Gazyvaro®), Elotuzumab (Empliciti) and Daratumumab (Darzalex®) for the treatment of hematological malignancies (REF); Avelumab (Bavencio®), Durvalumab (Imfinzi®); Olaratumab (Latruvo), Necitumumab (Potrazza), Atezolizumab (Tecentriq®), Dinutuximab (Unituxin), Ramucirumab (Cyramza), Denosumab (Xgeva®), Catumaxomab (Removab®) for the treatment of several solid tumors (**Table 2**).

Furthermore, some of these monoclonal antibodies are used for diagnostic purposes (Zhang et al., 2014) and others have been conjugated with cytotoxic molecules or radioactive isotopes, to specifically direct their high toxic activity against tumor cells (Beck et al., 2017; **Table 2**).

Other monoclonal antibodies developed since 2011 and directed against immune checkpoint inhibitors (Ipilimumab, Nivolumab, Pembrolizumab) will be discussed in more detail in the following chapter on immunotherapy.

Selective Tyrosine Kinase and Serine/Threonine-Protein Kinase Small Molecules Inhibitors

As stated before, several molecular pathways are altered in cancer because of gene mutations and protein modifications that lead to the abnormal activation of intracellular signal transduction,

such as MAPK and PI3K/Akt/mTOR, resulting in the increase of cell proliferation, reduced apoptosis, cell dedifferentiation, and cell migration (McCubrey et al., 2010). The study of these molecular alterations has led to the development of chemical small molecules able to selectively bind to molecular targets present in the tumor cells, causing their inhibition and cancer cell death by apoptotic mechanisms.

The selective inhibitors are generally divided into two main categories: selective tyrosine kinase inhibitors and intracytoplasmic serine/threonine kinase inhibitors (Wu et al., 2015). The new targets include growth factors, signaling molecules, cell-cycle proteins, modulators of apoptosis and molecules that promote angiogenesis. At the beginning of the '90s there was a growing industrial and scientific interest in developing new selective drugs for specific molecular targets known to be involved in cancer development; such growing interest has fostered both the efficacy of new cancer treatments and the economic development of pharmaceutical companies engaged in the development of anticancer drugs (Lange et al., 2014; Diaby et al., 2015).

The landmark event in the revolution of targeted therapy was represented by the development in the early '90s of the first selective tyrosine kinase inhibitor, Imatinib mesylate (Glivec®), a specific competitive inhibitor of ATP, directed to the fusion protein BCR-ABL typical of patients with chronic myelogenous leukemia (CML) and ALL that are Philadelphia chromosome-positive (Ph+). Before the discovery of Imatinib several compounds were obtained for the inhibition of BCR-ABL tyrosine kinase, among these Tyrphostins and similar compounds that displayed limited specificity (Anafi et al., 1993; Waller, 2018). Subsequently, in 1996, Buchdunger and colleagues synthesized a tyrosine kinase inhibitor selective against the ABL tyrosine kinase domain called 2-phenylaminopyrimidine or STI571 or Imatinib mesylate (Buchdunger et al., 1996; Druker et al., 1996). Further studies demonstrated that Imatinib, beside BCR-ABL, is also able to inhibit platelet-derived growth factor receptor (PDGFR) and mast/stem cell growth factor receptor (SCFR), also known as proto-oncogene c-Kit, frequently mutated in gastrointestinal stromal tumor (GIST) (Buchdunger et al., 2000; Tuveson et al., 2001).

Several clinical trials have demonstrated the efficacy and safety of Imatinib in patients affected by CML, ALL, and GIST. In particular, Imatinib is able to induce the complete remission in CML patients refractory to other treatments. The phase III IRIS clinical trial confirmed the efficacy of Imatinib in patients with CML in chronic phase (Hahn et al., 2003). Other clinical trials were performed to assess the efficacy of Imatinib against c-KIT and PDGF receptor in patients affected by advanced and/or metastatic GIST tumor showing a good response rate and consequently an increased overall survival (OS) (Dagher et al., 2002; Blanke et al., 2008). After Imatinib discovery, some studies showed that a percentage of patients were resistant *ab initio* to treatment with Glivec due to the presence of specific mutated variants of BRC-ABL, while some patients developed resistances during treatment (Milojkovic and Apperley, 2009). To cope with the poor efficacy of Imatinib treatment in this category of patients, new second and third generation BCR-ABL inhibitors were developed, including Dasatinib (Sprycel®), Nilotinib (Tasigna®), Bosutinib (Bosulif®) and Ponatinib (Iclusig®) (Rossari et al., 2018).

The second class of small molecules directed to tyrosine kinase proteins was represented by Gefitinib (Iressa®) and Erlotinib (Tarceva®) both directed to the EGFR ATP-binding site and able to inhibit the abnormal activation of MAPK and PI3K/AKT pathways overexpressed in cancer cells (Nicholson et al., 2001; Yarden, 2001).

The first anti-EGFR agent approved in 2001 for the treatment of NSCLC was Gefitinib, a potent and selective inhibitor of both EGFR and HER-2 kinases (Barker et al., 2001). In preclinical studies, Gefitinib demonstrated antitumor activity in several human cancer cell lines over-expressing EGFR, including lung, ovarian, breast, and colon cancer cell lines (Ciardiello et al., 2000). Currently, Gefitinib is used for the treatment of NSCLC. The first clinical trials recorded a partial remission in 10–15% of patients with NSCLC (Kris et al., 2003) although it showed reduced efficacy when administered in combination with other chemotherapeutic agents. The second EGFR selective inhibitor was Erlotinib, with a mechanism of action similar to Gefitinib. Erlotinib is approved for the treatment of NSCLC and for advanced and/or metastatic pancreatic carcinoma, in association with gemcitabine. Both Gefitinib and Erlotinib do not induce complete remission but do increase OS rate and limit tumor growth (Steins et al., 2018).

Another specific inhibitor of HER1 and HER2, approved in 2007, is Lapatinib (Tiverb®), developed against HER2 receptors. Lapatinib is able to bind the ATP-binding site of the HER2 receptor intracellular domain resulting in the inhibition of tumor cell growth. Through the introduction of targeted therapy, with both Trastuzumab and Lapatinib, the poor prognosis of HER2-positive cancer patients has been significantly ameliorated (Slamon et al., 2001). Currently, based on numerous clinical trials, Lapatinib is used in association with several chemotherapeutic agents, such as capecitabine (Geyer et al., 2006) or trastuzumab (Blackwell et al., 2012) in patients with advanced HER2-positive breast cancer. Despite the important clinical results obtained with Lapatinib, today it is used as a third or fourth line treatment, after more effective treatments, such as Trastuzumab-emtansine, or Neratinib, another irreversible pan-tyrosine kinase inhibitor (Voigtlaender et al., 2018).

Another class of tyrosine kinase inhibitors is represented by VEGF inhibitors, able to inhibit also other receptors, such as PDGFR, KIT and FLT3. The first two chemical small molecules synthesized and directed to the ATP binding pocket of VEGF receptor were Sunitinib (Sutent®) and Sorafenib (Nexavar®) (Ivy et al., 2009).

As anti-angiogenic drugs, both Sunitinib and Sorafenib are widely used in tumors for which few effective treatments are available and in advanced diseases after the failure of standard chemotherapy (Herrmann et al., 2008; Ivy et al., 2009). In particular, Sunitinib has been approved for the treatment of Imatinib-resistant GIST, renal carcinoma and neuroendocrine pancreatic tumors, while Sorafenib is indicated for the treatment of hepatocellular carcinoma, renal cell carcinoma and thyroid carcinoma (Imbulgoda et al., 2014; Hasskarl, 2018).

At the beginning of 2006, both FDA (January 2006) and EMA (July 2006) approved Sunitinib malate for the treatment of Imatinib-resistant GIST and advanced renal cell carcinoma. As mentioned, Sunitinib appears to inhibit multiple receptor tyrosine kinases by interfering with the ATP binding site. In particular, Sunitinib inhibits the activity of VEGF receptors 1 and 2, Kit, PDGFR-α, and –β, Fms-like TK-3 (FLT3); colony-stimulating factor receptor type 1 and neurotrophic factor receptor 7. By this way, Sunitinib is able to modulate tumor growth directly, by inhibiting the activation of signal transduction, and indirectly, by preventing tumor neo-vascularization (Adams and Leggas, 2007). Several *in vitro* studies showed the efficacy of Sunitinib in different tumor types, including colon, NSCLC, glioma, melanoma, etc. (Mendel et al., 2003). However, clinical trials showed Sunitinib more effective in patients with renal cell carcinoma and in those with acute myelogenous leukemia with FLT3 mutations (Fiedler et al., 2005; Motzer et al., 2006).

A few years before the approval of Sunitinib, Sorafenib, a small chemical molecule able to inhibit VEGF and PDGF receptors, was approved by the regulatory agencies. This drug was initially developed as a direct inhibitor of RAF-1 and BRAF intracytoplasmic serine/threonine kinases. Sorafenib was initially developed in 2001 (approved in 2004) by Bayer Pharmaceuticals as a selective inhibitor of both mutated and wild-type RAF, and *in vitro* showed a strong inhibitory power toward MAPKs pathway regulated by RAF. Subsequently, an inhibitory activity was also demonstrated for VEGFR1/2 and PDFGR in several *in vitro* models (Wilhelm et al., 2004). Further phase I, II, and III clinical trials showed the efficacy of Sorafenib in renal cell carcinoma patients, hepatocellular carcinoma patients, and thyroid cancer patients thanks to the multiple inhibition of VEGFR, PDGFR and BRAF (Strumberg et al., 2005; Gupta-Abramson et al., 2008; Llovet et al., 2008).

Other anti-angiogenic drugs are Aflibercept and Pegaptanib sodium, respectively a chimeric protein and an aptamer both directed against VEGF (Lytvynchuk et al., 2015).

A class of selective small molecules completely different from those discussed so far is that of mTOR inhibitors. mTOR is an intracellular serine/threonine kinase that plays a fundamental role in the regulation of gene expression and in the progression of the cell cycle from G1 to S phase. The first mTOR inhibitor was rapamycin derived from *Streptomyces hygroscopicus*. The two drugs currently used in the field of oncology, Temsirolimus (Torisel®) and Everolimus (Afinitor®) were derived from rapamycin (Sirolimus) and are still used in different tumor forms, such as renal cell carcinoma, mantle cell lymphoma, breast cancer and neuroendocrine pancreatic carcinoma (Fasolo and Sessa, 2012). mTOR was discovered by studying the mechanism of action of rapamycin, a macrolide antibiotic discovered in 1975 (Vézina et al., 1975). Rapamycin anticancer activities were defined in the '90s when it was found that it inhibited cellular proliferation and cell cycle progression by blocking mTOR/mTORC1 complex (Jayaraman and Marks, 1993; Carew et al., 2011).

Temsirolimus, previously named cell cycle inhibitor-779, is a soluble ester of rapamycin, identified in the '90s and subsequently used as an anticancer agent (Peralba et al., 2003). Everolimus, previously named RAD001, is a derivative of rapamycin able to bind FKBP12 and inhibit the mTORC1 complex, resulting in the down-regulation of the PI3K signal transduction pathway, which is frequently activated in human malignancies (Hasskarl, 2018). Both drugs showed great efficacy in the clinical trials. Temsirolimus showed anti-tumor activities toward several preclinical tumor models and in phase I–III trials for advanced renal cell carcinoma and mantle cell lymphoma (Neshat et al., 2001; Yu et al., 2001; Hudes et al., 2007; Ansell et al., 2008). More recently, Everolimus also showed a great anti-tumor activity both *in vitro* and in preclinical tumor models (O'Reilly et al., 2010). Several clinical trials have tried to assess the efficacy of Everolimus in hematological and solid tumors and some of them have provided encouraging results for its clinical use (Hasskarl, 2018).

Among the selective serine/threonine kinase inhibitors, BRAF inhibitors (Vemurafenib and Dabrafenib) and MEK inhibitors (Trametinib and Cobimetinib) are widely used in clinical practice for the treatment of mutated $BRAF^{V600E}$ melanomas, providing significant improvement in survival rates (Robert et al., 2015). Both Vemurafenib (Zelboraf® or PLX4032) and Dabrafenib (Tafinlar®) were approved after 2010 and are directed toward RAF protein, belonging to the RAS signal transduction pathway. The 15% of melanoma patients harbor RAS mutations and another 40–60% of patients are positive to the $BRAF^{V600E}$ mutations. Therefore, the discovery of selective BRAF inhibitors represented a turning point in the management of this aggressive form of tumor. Both Vemurafenib and Dabrafenib induce melanoma cell apoptosis by interfering with the B-Raf/MEK/ERK pathway. Despite several clinical trials demonstrated the efficacy of these treatments in patients positive for $BRAF^{V600E}$ mutation, resistance mechanisms limit the efficacy of the therapy in a high percentage of patients (Leonardi et al., 2018; Salemi et al., 2018).

To overcome these resistance mechanisms, BRAF inhibitors are generally combined with MEK inhibitors, such as Trametinib (Mekinist®) and/or Cobimetinib (Cotellic®) (Robert et al., 2015; Ascierto et al., 2016). Both Trametinib and Cobimetinib were approved in the last 5 years and are indicated for the treatment of BRAF mutant metastatic melanoma in order to avoid tumor relapse after surgical excision (Long et al., 2017).

Other selective inhibitors were developed to inhibit the proteasome machinery for the treatment of hematological malignancies, especially for multiple myeloma and mantle cell lymphoma. Among these inhibitors, Bortezomib (Velcade®) and Carfilzomib (Kyprolis®) are used in clinical practice; thanks to the inhibition of proteasome they prevent the degradation of pro-apoptotic factors, thus favoring the apoptotic death of cancer cells (Manasanch and Orlowski, 2017; Goldschmidt et al., 2018).

All these targeted therapeutic agents are currently used for the treatment of tumors, often in combination with other standard chemotherapeutic agents or in combination with monoclonal antibodies and/or other selective inhibitors (Vanneman and Dranoff, 2012). The availability of more drugs directed to different molecular targets has stimulated the development of different therapeutic strategies to make treatments more effective

and to overcome possible innate or acquired pharmacological resistance (Mokhtari et al., 2017).

IMMUNE CHECKPOINT INHIBITORS AS A NEW STRATEGY FOR CANCER TREATMENT

Cancer immunotherapy has experienced remarkable advances in recent years. After 2010, new monoclonal antibodies directed toward tumor antigens or T-cell protein receptors that downregulate the immune response have been developed (Haanen and Robert, 2015). These new drugs are defined immune checkpoint inhibitors and are monoclonal antibodies anti-cytotoxic T-lymphocyte-associated antigen 4 (anti-CTLA4) and anti-programmed cell death protein 1 antibody (anti-PD1), located in the membrane surface of T-cell and cancer cells, respectively (Seidel et al., 2018).

The first approved immune checkpoint inhibitor was Ipilimumab (Yervoy®), in 2011. This is a human IgG1 antibody that binds the membrane protein CTLA-4 expressed in regulatory T cells. The tumor microenvironment is able to induce the overexpression of CTLA-4 that binds the stimulating protein CD80 and CD86 present in the antigen presenting cells preventing their interaction with the T-cell surface receptor, responsible for the activation of immune system against cancer cells (Zitvogel et al., 2013).

Currently, Ipilimumab is used alone or in combination with Nivolumab for the treatment of unresectable or metastatic melanoma. The first clinical trials reported the improvement of long term-survival in melanoma patients with prolonged PFS and OS (Amdahl et al., 2016). Furthermore, several clinical trials are currently underway to establish the therapeutic efficacy of Ipilimumab, alone or in combination with Nivolumab, in other tumors as well as NSCLC, prostate cancer, renal cell carcinoma, etc. (Sakamuri et al., 2018).

More recently, two immune checkpoint inhibitor monoclonal antibodies were approved for the treatment of NSCLC, metastatic melanoma, NHL, and urothelial carcinoma as long as these tumors are positive to the presence of PD-L1. These two inhibitors are Nivolumab (Opdivo®) and Pembrolizumab (Keytruda®). Both drugs are human IgG4 anti-PD-1 antibodies directed toward the programmed cell death 1(PD-1) receptor of lymphocytes. This receptor, when linked to the PD-L1 antigens expressed from some tumors, is a down-regulator of T-cells, which become unable to recognize and kill cancer cells.

NSCLC patients treated with Nivolumab showed a lower risk of death and higher median PFS and OS compared to NSCLC patients treated with docetaxel (3-years PFS rate of 10% compared to <1%; 3-years OS rate 17 vs. 8% in patients treated with docetaxel; Vokes et al., 2018). These encouraging results are also confirmed by other clinical trials (Ramos-Esquivel et al., 2017).

Pembrolizumab has also shown therapeutic effects in patients with metastatic tumors, with limited side effects. Pembrolizumab is comparable to Nivolumab, suggesting a possible use of these two drugs as a first-line treatment for advanced or metastatic tumors (Brahmer et al., 2017; Fessas et al., 2017; Frenel et al., 2017).

Durvalumab (Imfinzi®), a human IgG1κ monoclonal antibody, blocks the interaction of programmed cell death ligand 1 (PD-L1) with the PD-1 and CD80 receptors. Durvalumab is approved for the treatment of patients with locally advanced or metastatic urothelial carcinoma (Faiena et al., 2018). Several studies are also evaluating its use for the treatment of patients with NSCLC (Antonia et al., 2017).

Finally, the immune checkpoint inhibitors are often administered in combination with each other or in combination with other chemotherapeutic agents, in order to make the treatment as effective as possible and to prolong the PFS and OS of the patients. The combination of anti-PD1 and anti-CTLA-4 inhibitors has shown a more durable response compared to monotherapy (Mahoney et al., 2015). The use of these drugs has revolutionized the treatment of incurable tumors, such as metastatic melanoma and NSCLC, increasing the life expectancy of patients and counteracting the onset of new metastases.

THE ROLE OF MOLECULAR RADIOTHERAPY IN CANCER

Molecular radiotherapy (MRT), called also unsealed source radiotherapy or unsealed source radionuclide therapy, is a well-known therapeutic approach used in clinical practice since many decades, based on the use of radioactive compounds (radiopharmaceuticals). Generally, radiopharmaceuticals are administered by ingestion or injection and their action is expressed toward the target cells recognized by specific carrier or depends on the radioisotope properties. The first report of the use of radiopharmaceuticals dates back to 1942 when Hertz used iodine-131 as a treatment for the autoimmune Basedow-Graves disease (Hertz et al., 1942). Nowadays, MRT is used for the treatment of both cancer and benign diseases by using simple radioactive compounds (e.g., sodium iodide) or recombinant antibodies labeled with radionuclides, specific for certain cells and tissues (Volkert and Hoffman, 1999; Buscombe and Navalkissoor, 2012).

MRT could also be considered a type of targeted therapy for the treatment of specific areas through the biological and radiopharmaceutical properties of the radiation treatment (Jadvar, 2017). In particular, the administration of 131I-Sodium Iodide for the treatment of thyroid cancers and 89Sr-Strontium chloride and 32P-Sodium phosphate for the treatment of bone metastasis are well-recognized treatments used since 1978 (Kutzner et al., 1978). In contrast to external beam radiotherapy, the use of systemic radiopharmaceuticals specifically localizes primitive and metastasized cancer cells, widely disseminated in the whole body, with minimal radiation-related damage to normal tissues (Choi, 2018). Since the 1980s, several radiopharmaceuticals were developed for treatment of cancers. These drugs were used alone or in combination with molecular carriers for enhancing their specificity toward cancer cells (Wilbur et al., 1996; Zhu et al., 1998). Thanks to the technological advances in the fields of molecular biology, genetic engineering

and chemistry it was possible to realize several conjugated drugs widely used in clinical practice. Among these, Iodine-131, MIBG (131I-MIBG metaiodobenzylguanidine), Radium-223 chloride, Strontium-89 chloride, Samarium-153 EDTMP, Phosphorus-32, Yttrium-90, and Yttrium-90 spheres were the most used drugs for both therapeutic and palliative purposes (Guerra Liberal et al., 2014; Jadvar, 2017).

Iodine-131 represents the first and most common radiopharmaceutical agents used for the treatment of thyroid cancers (Chung et al., 2010). It is composed by sodium iodide with a radioactive isotope of iodine. Its mechanism of action is based on the great affinity and uptake of iodide ion for the thyroid gland. This treatment is not only used for thyroid cancer pathologies but also for benign disease where the radiation emitted by radioiodine can have a beneficial effect (Silberstein et al., 2012). The beta radiations produced by sodium iodide determine damages to both normal and cancer thyroid cells inducing the cell deaths and thus having a therapeutic effect (Spitzweg et al., 2001).

Other unsealed radioactive sources are used as palliative treatments for the management of bone metastasis. Among these radioactive sources, Radium-223 chloride, Strontium-89 chloride, and Samarium-153 EDTMP are used for secondary bone metastatic disseminations of different cancer histotypes (Janjan, 1997; Choi, 2018). In particular, Strontium and Radium radioisotopes are taken up by bone as they mimic calcium ion, while samarium thanks to its covalent bond to tetraphosphate EDTMP is actively absorbed by osteoblasts, involved in the bone repair near the bone metastasis lesions (Wissing et al., 2013; Anderson et al., 2014). In these ways, Radium-223 chloride, Strontium-89 chloride, and Samarium-153 EDTMP can effectively counteract the progression of bone metastases, reduce patient suffering and prolong life expectancy.

Finally, other radiopharmaceuticals are used for the treatment of cancer, including Phosphorus-32, Yttrium-90 spheres for the treatment of colorectal liver metastasis, 131I-MIBG meta-iodo-benzylguanidine for the treatment of phaeochromocytoma and neuroblastoma, and Yttrium-90 and Lutetium-177 for the treatment of neuroendocrine tumors (Forrer et al., 2005; Sudbrock et al., 2010; Hadaki et al., 2011; Cheng et al., 2015).

NEW FRONTIERS IN THE TREATMENT OF CANCER

The drugs discovery in oncology is a constantly evolving field and every year several new approaches are proposed. As discussed above, after the Second World War there has been a rapid growth in the number of drugs available thanks to the important discoveries obtained in the biological, genetic and molecular fields. Parallel to the increase in the number of available drugs, there was also an increase in the effectiveness of the treatments, which consequently led to a significant improvement in the survival and quality of life of the patients.

Many clinical trials are currently underway to develop new drugs and therapeutic approaches for the treatment of hematological tumors and solid tumors. In particular, important results were obtained in the field of cell therapy, with the implementation of the so-called CAR-T cell therapy (Chimeric-Antigen Receptor) which led to the recent approval of two treatments, axicabtagene ciloleucel (Yescarta®) and tisagenlecleucel (Kymriah®), used respectively for the treatment of patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) and patients with relapsed/refractory B-cell precursor acute lymphoblastic leukemia (ALL) (Grupp, 2018).

CAR-T cell therapy consists in the ligation of engineered receptors to immune T cell specific for antigens expressed by the cancer cells, therefore the resulting chimeric T cell harbor a kind of monoclonal antibody with high specificity only toward cancer cells. In particular, the chimeric receptor is added to the immune cells by inserting a genic construct into the T cell DNA. The adjective "chimeric" means that the artificial receptors are constituted with protein structure derived from different DNA organisms and sources (Xu et al., 2018).

The realization of CAR-T cell therapy involves the removal of T-cells from the patient and their *in vitro* genetic modification for the addition of the chimeric receptor; subsequently, the engineered T-cells are reinfused into the patient, where they selectively interact with cancer cells, inducing immune-mediated cell death without affecting normal cells (Srivastava and Riddell, 2015). Potentially, CAR-T therapy can be implemented for all tumor types, a fundamental step for this possibility is to recognize specific antigens expressed by different tumors.

The first successful CAR-T therapy was developed against malignant B-cell responsible for a plethora of hematological tumors including acute ALL, chronic lymphocytic leukemia (CLL), and many different forms of Hodgkin's lymphoma. The axicabtagene ciloleucel (Axi-cel) therapy consist of chimeric T cells receptors against CD19, a surface molecule expressed in B-cells after their differentiation. Therefore, Axi-cel is effective for both normal and malignant B-cells determining their cell death, however, the B-cell precursor does not present CD19 antigen and for this reason is not affected by the treatment allowing the reconstitution of normal B-cells after treatment (Lee et al., 2015).

In 2010, Kochenderfer treated the first patient with an anti-CD19 CAR T therapy and obtained a significant clinical response (Kochenderfer et al., 2010). Subsequently, several clinical trials were performed to assess the efficacy and safety of anti-CD19 CAR-T. The most important clinical trial is the ZUMA-1 conducted on 22 patients with aggressive B cell lymphomas that showed an overall response rate in 73% of patients and a complete response in 55% (Kochenderfer et al., 2017). These results were confirmed by the phase II ZUMA-1 trials that demonstrated an overall response rate and a complete response in 82 and 54% of 101 total patients, respectively. However, some of these patients experienced acute toxicities and some of them died during the treatment (Neelapu et al., 2017).

The other currently approved CAR-T therapy for the treatment of B-cell acute lymphoblastic leukemia is the one using tisagenlecleucel. Similar to Axi-cel therapy, tisagenlecleucel also relies on artificial T cells with a chimeric anti-CD19 antigen. This therapy was developed by Carl June at the University of Pennsylvania and is a personalized treatment for the patient that is obtained with a 22-days experimental procedure. Via viral

vectors, the patient's T cells are modified by adding a chimeric gene coding for the specific CAR receptor for leukemic cells (Porter et al., 2011).

The clinical trials showed that CLL and ALL patients treated with Kymriah had a promising and durable antitumor efficacy with an 82% overall response rate and a complete response in 68% of patients (Mueller et al., 2017). Treatment with tisagenlecleucel is also associated with a series of adverse effects, among which the most important are the cytokine release syndrome and neurological events that require treatment in specialized centers (Badieyan and Hoseini, 2018). Many studies are trying to apply CAR-T therapy to solid tumors using modified heterologous cells obtained in cell factories.

Recently, many researchers are trying to develop new therapeutic approaches, based on genomic editing using CRISPR/Cas9 technology to correct genetic aberrations responsible for neoplastic transformation (Zhan et al., 2018).

Finally, in recent years, many research centers are developing therapeutic anticancer vaccines designed according to the individual characteristics of the tumor to make the immune system more active against the cancer cells and determine their death. However, the realization of these vaccines is complex due to the variability that characterizes each tumor. Already in 2008, the Oncophage vaccine was approved for the treatment of glioma, renal cancer, and metastatic melanoma; this vaccine consists of the heat shock protein 96 extracted directly from the tumor tissue and is supposed to stimulate the immune response against neoplastic cells of the same tumor (di Pietro et al., 2008). Subsequently, in 2010, another vaccine was approved, sipuleucel-T, for the treatment of metastatic, hormone-refractory, prostate cancer. This vaccine is again produced for each individual patient and consists of pulsed patient's dendritic cells with recombinant prostatic acid phosphatase expressed in the 95% of prostate cancer cells. In this way, the administration of the vaccine induces an increase in the immune response directed only to the tumor cells, determining their elimination (So-Rosillo and Small, 2006). Many other anticancer therapeutic vaccines are under study, but production difficulties make this approach particularly expensive and not suitable for all patients.

In conclusion, it is clear that cancer drug treatments are constantly evolving. From the Second Post-War to the advent of the new millennium, there has been an increase in the number of drugs and therapies available for the treatment of all hematological and solid tumors that have contributed to the significant reduction in cancer mortality rates. Furthermore, thanks to the primary and secondary prevention campaigns the reduction of incidence rates was recorded for many tumors, particularly for those of predominantly environmental etiology (**Figure 1**).

In the next few years, the development and approval of new highly innovative chemical, biological and biotechnological drugs are expected. These new treatments will start a new revolution in the field of clinical oncology, mainly based on a specific individual approach for each patient, a new personalized and more effective medicine.

AUTHOR CONTRIBUTIONS

LF and SS wrote the manuscript and were involved in data collection. LF has made the figures and tables. SS and ML conceived and reviewed the final version of the manuscript. All authors read and approved the final version of the manuscript.

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Drugs for Autoimmune Inflammatory Diseases: From Small Molecule Compounds to Anti-TNF Biologics

*Ping Li, Ying Zheng and Xin Chen**

State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macau, China

Although initially described as an anti-tumor mediator, tumor necrosis factor-alpha (TNF) is generally considered as the master pro-inflammatory cytokine. It plays a crucial role in the pathogenesis of inflammatory diseases, such as rheumatoid arthritis (RA), inflammatory bowel disease, ankylosing spondylitis (AS), and psoriasis. Consequently, anti-TNF therapy has become mainstay treatment for autoimmune diseases. Historically, anti-inflammatory agents were developed before the identification of TNF. Salicylates, the active components of *Willow* spp., were identified in the mid-19th century for the alleviation of pain, fever, and inflammatory responses. Study of this naturally occurring compound led to the discovery of aspirin, which was followed by the development of non-steroidal anti-inflammatory drugs (NSAIDs) due to the chemical advances in the 19th–20th centuries. Initially, the most of NSAIDs were organic acid, but the non-acidic compounds were also identified as NSAIDs. Although effective in the treatment of inflammatory diseases, NSAIDs have some undesirable and adverse effect, such as ulcers, kidney injury, and bleeding in the gastrointestinal tract. In the past two decades, anti-TNF biologics were developed. Drugs belong to this class include soluble TNF receptor 2 fusion protein and anti-TNF antibodies. The introduction of anti-TNF therapeutics has revolutionized the management of autoimmune diseases, such as RA, psoriatic arthritis (PsA), plaque psoriasis (PP), AS, CD and ulcerative colitis (UC). Nevertheless, up to 40% of patients have no response to anti-TNF treatment. Furthermore, this treatment is associated with some adverse effects such as increased risk of infection, and even triggered the *de novo* development of autoimmune diseases. Such harmful effect of anti-TNF treatment is likely caused by the global inhibition of TNF biological functions. Therefore, specific inhibition of TNF receptor (TNFR1 or TNFR2) may represent a safer and more effective treatment, as proposed by some recent studies. In this review article, the historical development of anti-inflammatory drugs after World War II as briefly described above will be reviewed and analyzed. The future trend in the development of novel TNF receptor-targeting therapeutics will be discussed in the context of latest progress in the research of TNF biology.

Keywords: tumor necrosis factor alpha (TNF), autoimmune inflammatory diseases, non-steroidal anti-inflammatory drugs (NSAIDs), anti-TNF biologics, TNF receptor

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Innsbruck Medical University, Austria

****Correspondence:***
Xin Chen
xchen@umac.mo

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INTRODUCTION

Autoimmune inflammatory diseases affect approximately 7.6–9.4% of the world population, especially among the young and middle-aged women (Cooper et al., 2009; Bragazzi et al., 2016). Frequently accompanied by severe and chronic morbidity, autoimmune diseases are also leading causes of death all around world. The patients with autoimmune inflammatory diseases need intensive medical intervention, which imposes the huge burden on public health service and economy (Bragazzi et al., 2016). Excessive and prolonged activation of immune cells, such as T and B lymphocytes, and overexpression of the master pro-inflammatory cytokine tumor necrosis factor alpha (TNF), together with other mediators such as interlukin-6 (IL-6), interlukin-1 (IL-1), and interferon gamma (IFN-γ), play a central role in the pathogenesis of autoimmune inflammatory responses in rheumatoid arthritis (RA), inflammatory bowel disease (IBD), Crohn's disease (CD), and ankylosing spondylitis (AS) (Moudgil and Choubey, 2011; Sticherling, 2016; Ellis and Braley-Mullen, 2017).

Non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying antirheumatic drugs (DMARDs) are traditionally used in the treatment of autoimmune inflammatory diseases. NSAIDs and glucocorticoids are effective in the alleviation of pain and inhibition of inflammation, while DMARDs have the capacity of reducing tissue and organ damage caused by inflammatory responses (Tabas and Glass, 2013). More recently, treatment for RA and other autoimmune diseases has been revolutionized with the discovery that TNF is critically important in the development of the diseases (Monaco et al., 2015). Anti-TNF biologics (such as infliximab, adalimumab, etanercept, golimumab, and certolizumab pepol) have markedly improved the outcome of the management of autoimmune inflammatory diseases (Meier et al., 2013). However, a considerable proportion of patients do not respond to anti-TNF treatment (Roda et al., 2016). Moreover, anti-TNF biologics are expensive, and are associated with some adverse effects. Recent studies indicate that specifically targeting of one of TNF receptors may represent a more effective and safer treatment for autoimmune disorders. In this review, the history of development of anti-inflammatory drugs, from small molecules to anti-TNF antibodies, will be discussed.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

The Historical Development of NSAIDs

Plant parts such as leaves of myrtle (Myrtus), bark of willow tree (Salix), bark of poplar (Populus), and meadow sweet (Spirea) were used in fork medicine for centuries in the treatment of fever, pain, and inflammatory responses (Hedner and Everts, 1998). These medicinal plants represented a primitive form of anti-inflammatory drugs. In the mid-19th century, salicylates were identified as active components of *Willow* spp. responsible for the anti-inflammatory activity, which laid the foundation for the mass synthesis of acetylsalicylic acid in 1899 (Vainio and Morgan, 1997; Vane, 2000). The progress in chemistry in the 19th–20th centuries promoted the fast development of NSAIDs. Initially, most of NSAIDs were the organic acid, but the non-acidic compounds were also discovered later. With outstanding safety profiles at dose ranges, ibuprofen was the first NSAIDs approved in the United Kingdom (Busson, 1986). After that, pharmaceutical companies began to develop NSAIDs with a series of chemical and biological properties (Rainsford, 2007). Overall, Post-World War II, the development of NSAIDs had experienced two periods: one was the pre-prostaglandin period (∼1970s) and another one was from 1970s to the end of the last century in which drugs were screened and evaluated partially based on their effect on the production of prostaglandin (Rainsford, 2007).

The Discovery of NSAIDs

Salicylic acid was synthesized by the Gerland in 1853 for the first time, and acetylsalicylic acid was synthesized by Charles Gerhardt in 1853 (Gerhardt, 1853; Gerland, 1853). Until 1876, salicylic acid was firstly used in clinic for the treatment of rheumatic disorders by two German physicians, Drs Streicher and Reiss (Hedner and Everts, 1998). Acetylsalicylic acid was rediscovered by Hoffman in 1897 (Hoffmann and Förster, 1987), and it became available worldwide in the treatment of rheumatic disorders and pain since then (Hedner and Everts, 1998). Acetylsalicylate was first used as Aspirin in 1899 (Vainio and Morgan, 1997). The development of aspirin, a prototype of NSAIDs, was a landmark (Vainio and Morgan, 1997), which was followed by the development of phenylbutazone (1946) and indomethacin (1963) (Shen, 1982). The term of 'non-steroidal anti-inflammatory drug' was used for the first time when phenylbutazone was introduced 3 years later as an anti-inflammatory agent. Thus, aspirin, phenylbutazone, and indomethacin were founding members of NSAID family.

The Categories of NSAIDs

Non-steroidal anti-inflammatory drugs have the analgesic, antipyretic, and anti-inflammatory effect, frequently used for the treatment of conditions like arthritis and headaches (Rainsford, 2007). NSAIDs relieve pain through blocking cyclooxygenase (COX) enzymes (Simmons et al., 2004). COX promotes the production of prostaglandins, a mediator which causes inflammation and pain (Simmons et al., 2004). Although NSAIDs have different chemical structures, all of them have the similar therapeutic effect, e.g., inhibition of autoimmune inflammatory responses (Rainsford, 2007). In general, NSAIDs can be divided into two broad categories: traditional non-selective NSAIDs and selective cyclooxygenase-2 (COX-2) inhibitors (Antman et al., 2007).

Traditional Non-selective NSAIDs

Based on the chemical structure, the traditional non-selective NSAIDs can be classified into different sub-types (Antman et al., 2007): (1) salicylic acid derivatives: acetylsalicylic acid (aspirin), diflunisal and sulfasalazine; (2) para-aminophenol derivatives: acetaminophen; (3) fenamates: mefenamic acid, meclofenamate, flufenamic acid; (4) propionic acid derivatives: ibuprofen,

naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin; and (5) enolic acid (oxicam) derivatives: piroxicam, tenoxicam. Most of these drugs were discovered in the pre-prostaglandins period and were developed in the 1960s. Their antipyretic, analgesic, and anti-inflammatory properties were discovered by animal studies, based on some biochemical experimental systems (Rainsford, 2007).

Selective COX-2 Inhibitors

The anti-inflammatory effect of NSAIDs is mainly based on the inhibition of activity of cyclooxygenase (COX) enzymes (Simmons et al., 2004). COX enzymes have two forms, e.g., COX-1 and COX-2. The COX-1 is constitutively expressed by the most of tissues and is responsible for the formation of prostaglandins and thromboxane A2, while the expression of COX-2 needs to be induced by inflammatory mediators (Antman et al., 2007). COX-1 plays an important role in some important physiological processes (Crofford, 1997). Therefore, complete inhibition of both COX-1 and COX-2 inevitably results in severe side effects (Cheng and Visco, 2012).

Inhibition of COX-1 by traditional non-selective NSAIDs causes various gastrointestinal toxicities. In order to reduce this harmful side effect, the selective COX-2 inhibitors were developed (Grosser et al., 2006). The selective COX-2 inhibitors can be further divided into two categories: selective COX-2 inhibitors and highly selective COX-2 inhibitors. The selective COX-2 inhibitors include meloxicam, salicylate, and nimesulide. These drugs more selectively inhibit COX-2, as compared to their action on COX-1. The highly selective COX-2 inhibitors characterized by replacing *cis*-stilbene with one of the pendant phenyl rings by different substitutes, termed as diarylheterocycles (Khanapure et al., 2003), including celecoxib, rofecoxib, valdecoxib, lumiracoxib, parecoxib, and etoricoxib (Antman et al., 2007). These coxibs have the same structure of diarylheterocycles which is decisively important for their highly potent inhibitory effect on COX-2 (Rainsford, 2007).

The Side Effects of NSAIDs

The traditional non-selective NSAIDs are able to inhibit both COX-1 and COX-2. These drugs also inhibit platelet aggregation and cause significant gastrointestinal disorders such as bleeding, ulcers, and perforation (Fujita et al., 2013). They also induce renal toxicity (Murray and Brater, 1993; Fujita et al., 2013). In contrast, the gastrointestinal adverse effect of selective COX-2 inhibitors is markedly reduced (Dajani and Islam, 2008). However, just like traditional non-selective NSAIDs, selective COX-2 inhibitor still have adverse effects on the cardiovascular system, including congestive heart failure, acute myocardial fraction, and even sudden death (Ray et al., 2002; Hermann and Ruschitzka, 2006).

GLUCOCORTICOIDS

The glucocorticoids were discovered by Hench and Kendal in 1940s (Hench et al., 1949), through an observation that cortisone had a significant beneficial effect for patients with severe RA (Glyn, 1998; Ferreira et al., 2016). The synthetic development and commercial efforts on glucocorticoids (or corticosteroids) were made in 1950–1980s, and consequently, a number of drugs were yielded (Neeck, 2002). Since then, the synthetic glucocorticoids are extensively used in the treatment of RA, asthma and other inflammatory diseases (Rhen and Cidlowski, 2005). These drugs include prednisone/prednisolone, methylprednisolone, and the fluorinated glucocorticoids such as dexamethasone and betamethasone, which are more frequently used (Buttgereit et al., 2011). Prednisone is an inactive pro-drug and its active metabolite is prednisolone (Frey, 1987). Usually, the prednisolone is administered once a day, while the duration of action of other glucocorticoids is longer when administered into patients with rheumatic diseases (Laev and Salakhutdinov, 2015). The binding of glucocorticoids to its specific receptor results in the inhibition of cellular signaling pathway such as AP-1 and NF-κB, and consequently regulate the expression of cytokines and chemokines (Barnes, 1998). We for the first time found that immunosuppressive $CD4^+Foxp3^+$ regulatory T cells (Tregs) were relatively more resistant to glucocorticoid-induced cell death (Chen et al., 2004). Furthermore, glucocorticoid selectively inhibited the proliferation of effector T cells (Teff) while promoting Treg proliferation induced by IL-2 (Chen et al., 2006). This property of glucocorticoid was harnessed to induce immune tolerance which may represent a novel approach in the treatment of autoimmune diseases (Kang et al., 2008).

Although the glucocorticoids are highly effective in the treatment of chronic diseases, they also induce severe side effects, including gastrointestinal ulcers and bleeding, infection, immunosuppression, and bone damage (Ethgen et al., 2013). Currently, low-doses of glucocorticoids are usually used in the treatment of autoimmune inflammatory diseases, while the adverse effects remain a major concern for prolonged usage (Curtis et al., 2006; Rainsford, 2007).

CONVENTIONAL DISEASE-MODIFYING ANTI-RHEUMATIC DRUGS (cDMARDs)

To relieve pain is a major goal in the management of RA and other arthritis. NSAIDs are able to alleviate pain and improve joint function through inhibition of inflammatory responses (Rainsford, 2007). However, NSAIDs alone do not reverse the pathological course of the autoimmune diseases (Tabas and Glass, 2013). Glucocorticoids can partially reduce pain symptom and suppress inflammation in the short-term (Laev and Salakhutdinov, 2015). Therefore, drugs that are capable of sustainably reducing inflammation with manageable side effects are highly desirable for the treatment of RA and other autoimmune diseases (Laev and Salakhutdinov, 2015). For this purpose, several drugs with different chemical structures, known as "disease-modifying anti-rheumatic drugs" (DMARDs), were developed.

Methotrexate

Methotrexate is an analog and antagonist of folic acid, which was synthesized firstly in 1947 (Swierkot and Szechinski, 2006). Methotrexate was firstly reported to be effective in the treatment

of RA in 1962 (Kersley, 1968), and after that it was also widely used as an anti-inflammatory drug for the management of other autoimmune diseases, including psoriasis, CD, and UC (Nathan et al., 2008; Yelamos and Puig, 2015). Methotrexate has been used as the first-line DMARDs since it was developed, due to its long-term effectiveness, satisfying responsive rate, acceptable toxicity profile, and low cost (Visser and van der Heijde, 2009; Favalli et al., 2014). Fundamental mechanisms underlying the therapeutic effect of methotrexate have been elucidated. For example, methotrexate was found to competitively inhibit the activity of folate-dependent enzymes, and synthesis of purine and pyrimidine which are required for DNA and RNA synthesis, and consequently suppress lymphocyte proliferation and production of pro-inflammatory cytokines (Meier et al., 2013). Nevertheless, the methotrexate monotherapy is no longer considered as the gold standard for RA treatment, for the reason that DMARDs combination therapy is more effective (Kremer et al., 2004).

Leflunomide

Leflunomide was approved for the treatment of RA in the 1998 (Gabriel et al., 2001). Leflunomide is an isoxazole derivative that is capable of blocking the rate-limiting enzyme dihydroorotate dehydrogenase, thereby selectively inhibits the synthesis of *de novo* pyrimidine ribonucleotides such as rUMP (Fox et al., 1999). Leflunomide is a pro-drug that is metabolized into teriflunomide *in vivo* (Laev and Salakhutdinov, 2015). Teriflunomide is a potent inhibitor of NF-κB activation, and consequently suppresses the production of pro-inflammatory cytokines. Leflunomide also reduces the production of metalloproteinases in synovial tissue and prevents joint destruction (Laev and Salakhutdinov, 2015). The results of meta-analyses strongly suggest that leflunomide is a DMARDs and effective in the treatment of RA (Osiri et al., 2003). As shown by several studies, the therapeutic effect of leflunomide is comparable to that of methotrexate (Silverman et al., 2005). Leflunomide can improve all clinical outcomes and delay radiographic progression at 6 and 12 months of RA patients (Sharp et al., 2000). Therefore, leflunomide should be considered as first-line therapy if contraindications to methotrexate are present. Furthermore, leflunomide is very useful in the combination therapy (Kalden et al., 2005).

Other DMARDs

Except for methotrexate and leflunomide, other DMARDs, include gold compounds, sulfasalazine, azathioprine, cyclophosphamide, antimalarials, D-penicillamine, cyclosporine, are all effective in the treatment of autoimmune inflammatory diseases such as RA (Gabriel et al., 2001). The DMARDs commonly used for the treatment of RA include hydroxychloroquine, sulfasalazine, methotrexate and leflunomide, while D-penicillamine, azathioprine, gold compounds, and cyclosporine are used less frequently (Kim et al., 2013).

Parenteral organic gold compounds have been used for RA treatment since 1920s (Simon, 2004), and their effect is comparable with other DMARDs (Meier et al., 2013). Nonetheless, the toxicity of gold compounds, especially the adverse effect on skin and mucosa, is a major concern which limits their clinical application (Bendix and Bjelle, 1996).

Sulfasalazine was initially investigated for the treatment of IBD, including UC and CD (Box and Pullar, 1997). This drug was firstly reanimated in Europe where it has been used in early RA patients. The exact mechanism of sulfasalazine is still unclear, although it inhibits the production of pro-inflammatory cytokines, including TNF and IL-6 (Wahl et al., 1998). The main side effect of sulfasalazine is gastrointestinal upset or rash (Skosey, 1988).

Antimalarial drugs hydroxychloroquine and chloroquine have the capacity to inhibit the clinical progress of several autoimmune diseases, including RA and systemic lupus erythematosus (SLE) (Taherian et al., 2013). Although the exact molecular mechanism of its immunosuppressive effect remains to be fully understood, both drugs can inhibit the activation of T cells, B cells (Taherian et al., 2013) and the production of pro-inflammatory cytokines such as TNF, IL-6, and IL-1β (Jang et al., 2006). Both drugs almost have the same side effects, such as rare irreversible retinopathy and a more rarely form of myopathy (Costedoat-Chalumeau et al., 2015).

Combination Therapy with cDMARDs

Among cDMARDs drug class, the methotrexate is still an 'anchor drug' for RA therapy, and the first choice for the most of DMARD-naïve patients (Favalli et al., 2014). However, a considerable proportion of patients do not respond to methotrexate. Either anti-TNF biologics (which will be discussed later) or a combination therapy of cDMARDs should be considered for those patients (van Vollenhoven et al., 2012). Methotrexate has been used in combination with other drugs, including sulfasalazine, sulfasalazine and hydroxychloroquine, cyclosporine and biologic agents such as etanercept and infliximab (Kremer et al., 2004; Swierkot and Szechinski, 2006). For example, the concomitant treatment of methotrexate and leflunomide results in a synergistic effect in the treatment of patients with RA (Boers et al., 1997; Kremer et al., 2004). The triple combination of methotrexate, sulfasalazine and hydroxychloroquine represent an effective therapy regimen since 1996 (Meier et al., 2013), which is more effective and safer than the monotherapy with methotrexate, or methotrexate combination of sulfasalazine, or hydroxychloroquine (ODell et al., 1996, 2002). It has been reported that the combination of methotrexate and cyclosporine, or leflunomide and cyclosporine, are more effective than each drug alone (Karanikolas et al., 2006). However, the toxicity of cyclosporine, including reversible and irreversible renal disease, hypertension and heurism, remains an important issue in the combination therapy (Gabriel et al., 2001). Therefore, the ideal cDMARDs combination therapy should be one that is synergistic in their efficacy while lacking additive effects of toxicity.

ANTI-TNF BIOLOGICS

In 1975, Lloyd and colleagues discovered an endotoxin-inducible serum factor named TNF had the capacity to cause necrosis of tumors (Carswell et al., 1975). It is one of subsequent studies of "Coley's toxin," a therapeutic regimen using killed bacteria

to induce tumor regression in cancer patients, invented by Dr. William Coley in 1891 (Coley, 1891). Although initially identified as anti-tumor molecule, TNF is now considered as a pleiotropic cytokine which plays a major role in immune or inflammatory responses (Palladino et al., 2003; Efimov et al., 2009).

Feldman's group showed that a number of pro-inflammatory cytokines such as IL-1, TNF, IL-6, GM-CSF, and IFN-γ were expressed at a high levels in synovium samples from active RA patients (Feldmann et al., 1996). Intriguingly, anti-TNF antibody inhibited the expression of other pro-inflammatory cytokines (IL-1, IL-6, and GM-CSF) in the synovial culture (Feldmann et al., 1996). Furthermore, anti-TNF antibodies such as infliximab and adalimumab suppressed antigen-induced IFN-γ production in blood (Wallis, 2007). These results provide the evidence that TNF plays a predominant role in the pathogenesis of autoimmune inflammatory diseases. IFN-γ is also an important mediator in the inflammatory responses, such as Schwartzman reactions (Billiau, 1988) and collagen-induced arthritis (Mauritz et al., 1988). It was also reported that IFN-γ promoted the production of TNF induced by LPS stimulation (Billiau, 1988). However, unlike the effect of anti-TNF biologics, treatment with antibody against IFN-γ did not markedly ameliorate the arthritic symptoms in RA patients (Sigidin et al., 2001; Schurgers et al., 2011).

TNF is now generally considered as a master pro-inflammatory cytokine that plays a critical role in the pathogenesis of autoimmune inflammatory diseases (Moudgil and Choubey, 2011). Consequently, anti-TNF biologics, which are designed to block the biological function of TNF, have been developed for the therapy of autoimmune inflammatory diseases (Meier et al., 2013). In the past two decades, five TNF-targeting drugs have been approved for clinical use, including infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol, for the treatment of the autoimmune inflammatory diseases including RA, CD and AS (Monaco et al., 2015).

Infliximab

Infliximab is a recombinant chimeric antibody that is generated by mouse myeloma cells. The structure of infliximab contains a constant sequence of human IgG1 and variable regions of murine, which is specific for all forms of TNF in human and chimpanzees, and effectively blocks the binding of TNF to its soluble and membrane receptors (Monaco et al., 2015). The maximum serum concentration of infliximab can be reached within 1 h by intravenous administration (Atzeni et al., 2005), and the half-life is approximately 8–10 days (Chatzantoni and Mouzaki, 2006). After that, infliximab can be maintained by dosing every 8 weeks. Infliximab is capable of mediating both complement-dependent and antibody-dependent cytotoxicity on a cell line overexpressing TNF (Tracey et al., 2008). Infliximab is initially approved for the treatment of Crohn's disease by the US Food and Drug Administration (FDA) and later also for RA. It was further approved for the treatment of AS, PsA, UC, CD, and chronic plaque psoriasis (Atzeni et al., 2005).

Etanercept

Etanercept comprises soluble TNF receptor 2 and Fc portion of IgG1, which is a human recombinant protein (Kerensky et al., 2012). After subcutaneous administration, etanercept has a half-life of 3–3.5 days (Chatzantoni and Mouzaki, 2006). Etanercept binds to and inactivates soluble form and membrane form of TNF and lymphotoxin, by blocking their interaction with the receptors (Tracey et al., 2008). Similar as infliximab, etanercept alleviate the signs and symptoms of arthritis and inhibits progress of RA in patients (Moreland et al., 1999). Furthermore, it is more effective when combined with methotrexate (Emery et al., 2008). Etanercept is also approved by FDA for the treatment of juvenile rheumatoid arthritis and PsA, RA, PP, and AS (Kerensky et al., 2012).

Adalimumab

Adalimumab is a fully humanized IgG1 monoclonal antibody, which can specific block human TNF binding to its receptors (Atzeni et al., 2005). Adalimumab needs a less frequent subcutaneous injection because of its relative longer half-life which is about 2 weeks (Simon, 2004). Furthermore, adalimumab shows the lower immunogenicity than infliximab (Rau, 2002).

Adalimumab is effective in alleviating the signs and symptoms of moderate to severe RA patients (van de Putte et al., 2004). Adalimumab is also effective in the treatment of patients with CD, with good tolerance and minimal immunogenicity, thus can be used in patients with allergic reactions to infliximab (Sandborn et al., 2004). Adalimumab has been approved by FDA for the treatment of autoimmune diseases including RA, PsA, AS, and CD (Lapadula et al., 2014).

Golimumab

Golimumab is also a fully humanized IgG1 monoclonal antibody, which has a high affinity and specificity for human TNF (Shealy et al., 2007). As shown by the preclinical data, golimumab has higher affinity and is more effective in the neutralization of soluble and trans-membrane forms of TNF, as compared with infliximab and adalimumab, thus it can potently neutralize TNF biological activity (Shealy et al., 2010). The half-life of golimumab is about 7–20 days (Zhou et al., 2007). Golimumab was approved by the FDA in 2009 for the treatment of moderate-to-severe RA when administrated in combination with methotrexate (Mazumdar and Greenwald, 2009). It was also approved for UC treatment by FDA and European Medicines Agency (EMA) in 2013 (Lowenberg and D'Haens, 2013).

Certolizumab Pegol

Certolizumab pegol (CDP870) is a humanized monoclonal antibody with the structure of polyethylene glycolated Fab fragment, which is a novel TNF inhibitor with a distinct mechanism of action compared with other TNF inhibitors (Pasut, 2014). The unique structure might be an explanation for certolizumab pegol with higher efficiency in comparison with other TNF inhibitors (Desai et al., 2012). PEGylation of certolizumab pegol improves its half-life to 2 weeks which may

contribute to its high concentration in the inflamed tissues (Keystone et al., 2008).

Certolizumab pegol monotherapy could effectively control the symptoms of patients with RA (Choy et al., 2002). It is also effective the treatment of patients with moderate to severe CD (Schreiber et al., 2005). Certolizumab pegol has been approved for the treatment of CD and PsA by FDA (Deeks, 2016).

Biosimilars

Biosimilar has been defined as a biological medicinal product that has the similar quality, safety, and efficacy with the already approved biological medicine (Fiorino and Danese, 2014). Anti-TNF biosimilars that are extensively developed in recent years will help reduce the cost of anti-TNF treatment. To date, a number of anti-TNF biosimilar products (summarized in **Table 1**) have been marketed (Dorner and Kay, 2015). CT-13 is the first biosimilar monoclonal antibody against TNF. Comparing with the original TNF inhibitor infliximab, CT-13 is equally effective and safe (Yoo et al., 2013). It is approved in European for the treatment of RA, AS, PsA, PS, CD, and UC (Dorner et al., 2016).

The Side Effects of Anti-TNF Biologics

Although anti-TNF biologics are generally effective in the treatment of patients with different autoimmune inflammatory diseases (Willrich et al., 2015), not all patients respond equally well to the treatment. Up to 40% of patients have no response to anti-TNF treatment (Roda et al., 2016). Furthermore, several types of adverse effects have been associated with anti-TNF biologics.

It was reported recently that PBMCs isolated from patients responding to the treatment of adalimumab and etanercept can produce higher levels of TNF and soluble TNFR2 (sTNFR2) than those from patients responding to infliximab, and PBMCs isolated from patients who do not respond to infliximab produce higher levels of TNF and sTNFR2 than those from patients responding to infliximab (Gibellini et al., 2016). Therefore, anti-TNF biologics may have major and different effects on TNF expression and soluble TNFR2 levels in patients. It was reported that the TNF gene polymorphism (TNF-alpha 308G > A) is association with patients who do not respond to anti-TNF therapy (Wijnen et al., 2014). Furthermore, the polymorphism of tumor necrosis factor receptor superfamily member 1B, a gene encoding TNFR2 protein, is able to predict responses of patients with autoimmune diseases to anti-TNF therapy (Chen W. et al., 2015). Thus, aberrant expression of TNF and TNFR2, and their responses to TNF blockade, is responsible at least partially for the outcome of anti-TNF therapy.

TNF plays a crucial role in host defense to invading pathogens (Pfeffer, 2003). Anti-TNF biologics can inhibit IFN-γ expression in the blood (Wallis, 2007). In addition, TNF is a good inducer of nitric oxide (NO) in macrophage, and TNF also synergizes with IFN-γ in inducing inducible nitric oxide synthase (iNOS) (Vila-Del Sol et al., 2007), while iNOS is able to synthesize antimicrobial nitric oxide (Fang, 1997). This can explain why infections, such as tuberculosis and pneumonia, are common adverse event of patients treated with anti-TNF biologics (Antoni and Braun, 2002).

There is also some evidence that anti-TNF treatment is associated with increased risk of malignancy (Bongartz et al., 2006). However, this is not supported by the more recent studies (Minozzi et al., 2016; Shelton et al., 2016). In fact, the underlying inflammatory condition is likely to promote the development of cancer, while anti-inflammatory treatment has the beneficial effect in inhibition of malignancy (Lasry et al., 2016). For example, patient with RA and CD has a higher risk of lymphoma compared with the general population (Antoni and Braun, 2002). Therefore, patients with malignancy after treatment with anti-TNF biologics should be more carefully studied.

Anti-TNF treatment has been tried in patients with congestive cardiac failure, since the high levels of circulating TNF was found in such patients. However, infliximab, adalimumab, and etanercept have deleterious, rather than beneficial, effects on congestive cardiac failure patients (Balakumar and Singh, 2006). Anti-TNF biologics have been reported to have side effects on the neurological system. For example, anti-TNF treatment exacerbated diseases in almost all multiple sclerosis (MS) (Robinson et al., 2001). Paradoxically, some new autoimmune diseases can be *de novo* induced by anti-TNF therapy. For example, psoriasis can be induced in IBD patients treated with anti-TNF biologics (Guerra et al., 2012). Therefore, more effective and safer TNF-targeting treatment needs to be developed. Thorough understanding of the fundamental biology of TNF and it receptors is a prerequisite to reach this goal.

ANTI-TNFR THERAPEUTICS: NEXT GENERATION OF TNF-TARGETING DRUG?

Tumor necrosis factor-alpha is a pleiotropic cytokine that is involved in the initiation and orchestration of inflammation and immunity (Aggarwal, 2003). Two receptors of TNF, namely TNFR1 (P55) and TNFR2 (P75), mediate different signaling pathways and induce diverse biological effects of TNF (Vandenabeele et al., 1995). TNFR1 is expressed by almost all cell types except erythrocyte, whereas TNFR2 is more strictly expressed by immune cells. The capacity of TNF to induce cell death is mediated by TNFR1. Conversely, TNFR2 signaling triggers cell survival pathways and promotes cell proliferation (Faustman and Davis, 2010). Initially, TNF is expressed on the cell surface as a transmembrane protein. It is then cleaved by a metalloprotease, called TNF-alpha converting enzyme (TACE) (Black et al., 1997). This process liberates a trimeric soluble protein, namely soluble TNF (sTNF). Both membrane-bound TNF and soluble TNF can bind to TNFR1 and TNFR2, but membrane-bound TNF preferentially binds with TNFR2 (Grell et al., 1995).

There is now compelling evidence that TNFR2 is constitutively expressed by immunosuppressive $CD4^+Foxp3^+$ regulatory T cells (Tregs) and TNF-TNFR2 interaction preferentially activates and expands naturally occurring Tregs (Chen et al., 2007, 2008, 2010, 2013; Nguyen and Ehrenstein,

TABLE 1 | Anti-TNF biosimilars in the development for the treatment of autoimmune inflammatory diseases.

Anti-TNF biologics	Biosimilar	Indicates	Current development stage in May 2017	Sponsor
Infliximab	ABP 710	RA	Phase III in RA	Amgen
	BCD-055	RA, Psoriasis, AS	Phase III in RA, Psoriasis Phase I completed in AS	Biocad
	BOWO15	RA	Phase III in RA	Epirus Biopharmaceuticals (Switzerland) GmbH
	CT-P13	RA,CD,AS	Phase III completed in CD, RA Phase III completed in AS	Celltrion
	NI-071	RA	Phase III completed in RA	Nichi-Iko Pharmaceutical Co., Ltd.
	PF-06438179	RA	Phase III in RA	Pfizer
	SB2	RA	Phase III completed in RA	Ohio State University Comprehensive Cancer Center
Etanercept	CHS-0214	RA,PP	Phase III completed in RA and PP	Coherus Biosciences, Inc.
	ENIA11	RA	Phase III in RA	TSH Biopharm Corporation Limited
	GP2015	RA,PP	Phase III completed in RA, Chronic Stable Plaque Psoriasis	Sandoz
	HD203	RA	Phase III completed in RA	Hanwha Chemical
	LBEC0101	RA	Phase III in RA	LG Life Sciences
	SB4	RA	Phase III completed in RA	Samsung Bioepis Co., Ltd.
Adalimumab	ABP 501	RA, Psoriasis	Phase III completed in RA and Psoriasis	Amgen
	BCD-057	Psoriasis	Phase III in Psoriasis	Biocad
	BI 695501	RA	Phase III completed in RA	Boehringer Ingelheim
	CHS-1420	PP	Phase III completed in PP	Coherus Biosciences, Inc.
	GP2017	RA, Psoriasis	Phase III completed in Plaque Type Psoriasis Phase III in RA	Sandoz
	LBAL	RA	Phase III in RA	LG Life Sciences
	M923	RA	Phase III completed in RA	Momenta Pharmaceuticals, Inc.
	PF-06410293	RA	Phase III in RA	Pfizer
	MSB11022	RA, PsA, PP	Phase III in RA, PsA, PP	EMD Serono Research & Development Institute, Inc.
	SB5	RA	Phase III completed in RA	Samsung Bioepis Co., Ltd.
Golimumab	BOW100	AS,PsA,RA,UC	Preclinical	Bioceros
	ONS-3035	RA, UC	Preclinical	Oncobiologics
Certolizumab pegol	PF688	RA, CD	Preclinical	Pfenex
	Xcimzane	RA, PsA, UC, AS	Preclinical	Xbrane

TABLE 2 | Alternative biologic in autoimmune inflammatory diseases.

Drug	Target	Type of molecules	Indications	Reference
Abatacept (Orencia)	CD80 (B7-1) and CD86 (B7-2) and blocks activation of T-cell Ab4Ig	The extracellular domain of CTLA4 and Fc domain of IgG1	RA, JIA, SLE	Genovese et al., 2005
Tocilizumab (Actemra)	IL-6	A humanized anti-human IL-6 receptor monoclonal antibody	RA after treatment failure with TNF inhibitors	Okuda, 2008
Anakinra (Kineret)	IL-1	A recombinant human IL-1 receptor antagonist	RA	Fleischmann et al., 2003
Ustekinumab	IL-12 and IL-23	Human IgG1k mAb	Psoriasis	Koutruba et al., 2010
Rituximab	CD20	A chimeric murine/human monoclonal IgG1k antibody	RA, SLE	Fleischmann, 2009
Secukinumab	IL-17	A human IgG1κ monoclonal antibody	PP	Campa et al., 2016

CTLA, cytotoxic T lymphocyte-associated; JIA, juvenile idiopathic arthritis; SLE, systemic lupus erythematosus.

2016). The immunosuppressive property of TNFR2 has been shown by many studies. For example, TNFR2 agonist is able to promote the expansion of Tregs more potently than TNF itself, and consequently inhibits autoimmune responses (Okubo et al., 2013). Selective blockade of TNFR1 also results in the proliferative expansion and activation Tregs (McCann et al., 2014), and inhibits clinical symptoms in CIA model (Shibata et al., 2009). It was shown recently that therapeutic

anti-TNF antibody Infliximab binds to and promotes the expression of membrane bound TNF on monocytes from RA patients, and consequently enhances Treg activity through TNF-TNFR2 interaction (Chen and Oppenheim, 2016; Nguyen and Ehrenstein, 2016). These evidences favor the idea that biologic drugs which can selectively inhibit TNFR1, or selectively activate TNFR2, may be more effective and safer than those globally inhibit TNF. The expression of TNFR2 on cells and tissues is more limited than TNFR1. Thus, therapeutically targeting TNFR2 may have fewer side effects than targeting TNFR1. Expression of TNFR2 on $CD4^+Foxp3^-$ effector T cells (Teffs) can be induced and upregulated by TCR stimulation, and TNFR2 expression on Teff cells has a functional consequence (Chen and Oppenheim, 2011; Govindaraj et al., 2013; Chen X. et al., 2015; Chen and Oppenheim, 2016; Chen et al., 2016; Zaragoza et al., 2016). Thus, the effect of TNFR2 on Teff function should be considered in the development of TNFR2-targeting therapeutics.

OTHER BIOLOGICAL DRUGS FOR AUTOIMMUNE INFLAMMATORY DISEASES

In addition to anti-TNF agents, the biologics targeting other proinflammatory cytokines or immune competent molecules have also been extensively studied and actively developed. For example, abatacept, a fully humanized fusion protein of extracellular domain of CTLA-4 and Fc fraction of IgG1, has been approved for the RA patients with inadequate response to anti-TNF therapy (Genovese et al., 2005). The major immunological mechanism of abatacept is selective inhibition of co-stimulation pathway (CD80 and CD86) and activation of T cells. Tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody was approved for RA patients intolerant to DMARDs and/or anti-TNF biologics (Okuda, 2008). This therapeutic mAb blocks the transmembrane signaling of IL-6 through binding with soluble and membrane forms of IL-6 receptor. Biological drugs targeting IL-1 (anakinra), Th1 immune responses (IL-12/IL-23, ustekinumab), Th17 immune responses (IL-17, secukinumab) and CD20 (rituximab) have also been approved for the treatment of autoimmune diseases, as summarized in the **Table 2**, as a complementary and alternative biological treatment of anti-TNF therapy.

CONCLUSION

Although TNF plays a central role in acute and chronic inflammation, the anti-inflammatory agents were developed before the identification of TNF. After the World War II, agents from small molecules to anti-TNF antibodies have been developed, including NSAIDs, glucocorticoids, DMSADs, and anti-TNF biologics. The traditional non-selective NSAIDs are associated with severe gastrointestinal disorders, attributable to simultaneous inhibition of COX-1 and COX-2, which prompted the development of selective NSAIDs. Glucocorticoids have potent anti-inflammatory activity, accompanied by some severe adverse effects. Methotrexate remains the first-line drug, while the efficacy can be enhanced and adverse effects can be reduced by DMARDs combination therapy. Currently, five anti-TNF biologics have been approved for patients with autoimmune disorders. With great success in treating autoimmune diseases, anti-TNF biologics also represent the most profitable drug class in the history, exceeding $US 25 billion total sale in 2012 (Monaco et al., 2015). Anti-TNF biosimilars bring the hope to reduce the medical costs and consequently improve the accessibility to this revolutionized treatment. Nevertheless, the effectiveness and safety of TNF-targeting treatment should be further improved, hopefully through the selective blockade of TNFR1 or activation of TNFR2. For those patients failed to respond appropriately to anti-TNF treatment, biologics targeting other pro-inflammatory cytokines and pathways provide an alternative therapy.

As compared to small molecule compounds, biologics have some clear advantages, such as higher safety profile or minimal toxicity, well-understood mechanisms, and more importantly, the target specificity (Tracey et al., 2008; Meier et al., 2013; Monaco et al., 2015). Due to these advantages, the biologics, including therapeutic monoclonal antibodies and antibody-drug conjugate (ADC), have become main stream therapeutics (Meier et al., 2013; Monaco et al., 2015). This class of drug represents the fastest growing segment of global pharmaceutical marked (based on the data of global sale and FDA approval) (Ioannidis et al., 2013; Monaco et al., 2015). Therefore, it is predictable that the development of new biologics for the treatment of autoimmune diseases will be the focus in the future, which will be facilitated by the more thorough understanding of molecular basis of autoimmune diseases.

AUTHOR CONTRIBUTIONS

PL drafted the work; YZ and XC revised the manuscript. PL, YZ, and XC contributed substantially to the conception or design of the work, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Innovating Chinese Herbal Medicine: From Traditional Health Practice to Scientific Drug Discovery

Shuo Gu[1,2] *and Jianfeng Pei*[1]*

[1] Center for Quantitative Biology, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China, [2] Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, United States

As one of the major contemporary alternative medicines, traditional Chinese medicine (TCM) continues its influence in Chinese communities and has begun to attract the academic attention in the world of western medicine. This paper aims to examine Chinese herbal medicine (CHM), the essential branch of TCM, from both narrative and scientific perspectives. CHM is a traditional health practice originated from Chinese philosophy and religion, holding the belief of holism and balance in the body. With the development of orthodox medicine and science during the last centuries, CHM also seized the opportunity to change from traditional health practice to scientific drug discovery illustrated in the famous story of the herb-derived drug artemisinin. However, hindered by its culture and founding principles, CHM faces the questions of the research paradigm posed by the convention of science. To address these questions, we discussed two essential questions concerning the relationship of CHM and science, and then upheld the paradigm of methodological reductionism in scientific research. Finally, the contemporary narrative of CHM in the 21st century was discussed in the hope to preserve this medical tradition in tandem with scientific research.

Keywords: Chinese herbal medicine, traditional Chinese medicine, drug discovery, medical narrative, methodological reductionism

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Apostolos Zarros, Queen Mary University of London, United Kingdom

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Alice L. Perez, University of Costa Rica, Costa Rica
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Honglin Li, East China University of Science and Technology, China

***Correspondence:**
Jianfeng Pei
jfpei@pku.edu.cn

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INTRODUCTION

The Philosophy and Ancient Narrative of Chinese Herbal Medicine

Traditional Chinese Medicine has a history of about 3000 years starting from the early Zhou Dynasty of China or even earlier as the oldest medical writings on herbs were found in *Classic of Changes* (*Yi Jing*) and *Classic of Poetry* (*Shi Jing*) (Reid, 1996). In these classics, dozens of herbs were mentioned in a variety of situations related to healing and diet. Later, TCM evolves into an independent discipline as accumulated knowledge was documented in medical books. Among which, the most famous four classics are *Inner Canon of the Yellow Emperor* (*Huang Di Nei Jing*, ∼26 BCE), *Yellow Emperor's Canon of Eighty-One Difficult Issues* (*Nan Jing*, ∼106 CE), *Treatise on Cold Damage Disorders* (*Shang Han Lun*, ∼206 CE), and *Shennong's Materia Medica* (*Shen Nong Ben Cao Jing*, ∼220 CE). These classic works constitute the early foundation of TCM.

While there are different branches of TCM like Chinese herbal medicine, acupuncture, and Qigong, the essential philosophy is the same. Western people might be most familiar with the metaphysics of Yin and Yang, representing the two ends of a spectrum like cold-hot, female-male,

and inside-outside. When this concept is applied to the human body, Yin and Yang are linked to different parts or organs of the body, or simply one's feeling of cold and hot. Consistent with the philosophy of Taoism, one is expected to keep balance of his Yin and Yang; otherwise the breaking of balance gives rise to different syndromes or diseases. In such cases, herbs with "hot" properties can be used to treat a "cold" syndrome, and vice versa (Chan, 1995).

Another fundamental theory in TCM is the Five Elements theory, which describes the human body and herbs with five elemental qualities (Wood, Fire, Earth, Metal, and Water). For example, Water represents kidney, bladder, and ears, while Fire represents heart, small intestine, and tongue. Furthermore, since the five elements are associated in both the generating circle (Wood -> Fire -> Earth -> Mental -> Water -> Wood) and the overcoming circle (Wood -> Earth -> Water -> Fire -> Mental -> Wood), all the organs in the body are also connected and can be affected according to the attributes of certain herbs (Chan, 1995).

Before the 20th century, most Chinese could only seek TCM as a medical service, in which herbal medicine was intensively used. The history of Chinese herbal medicine (CHM) is largely associated with the creative yet appealing narratives of healing. The narrative tradition, either in the form of classics or passed down orally, preserves the medical knowledge of ancient Chinese people and continues to strengthen the belief in herbal healing in the Chinese communities to this day. It appears that the majority of Chinese still believe in the power of CHM, although orthodox medicine is popular and affordable in China now. Similar to a religion, CHM in China is almost like a faith in the herbal materials that can cure disease and save life.

Then, how did the ancestors of Chinese link various herbs to certain diseases? One possible reason could be the doctrine of signatures that Chinese people tend to relate the efficacy of a herb to its name, shape, or other figurative notions (Bennett, 2007). For example, ginseng in Chinese means "essence of men." People also call it "earth elf" because the shape of a ginseng root resembles a little man. In the medical usage, only the root is chosen as the medicinal material because the ancient Chinese believed this figurative part to help restore the inner energy of the human body (Hu, 1977). Moreover, the older the ginseng root, the better quality it is assumed as the longevity of a man-shaped herb can increase the life expectancy of the consumer. Although scientific findings do not necessarily support the argument, this traditional belief is quite popular in China. Put another way, the ancient narratives of CHM dominate one's understanding toward herbs and consequently strengthen the belief in herbal healing.

In Comparison with the Treatments in Medieval Medicine of Western Europe

The Medieval medicine of western Europe originated from ancient Greek medicine. Greek people believed that the universe is composed of four elements (fire, air, water, and earth), while the human body is also made of these: fire for liver, air for heart, water for brain, and earth for spleen. Meanwhile, there are four primal qualities (warm, cold, dry, and moist) describing the attributes of each element, e.g., warm and dry for fire.

In one medieval medical book *Causes and Cures* written by Hildegard of Bingen, we can find interesting treatments whose logic is quite similar to that of CHM. "If persons cannot hold urine due to the coldness of their stomachs, they should frequently drink wine that has been heated over fire, mix vinegar into all their foods and drink vinegar as often as possible. This way the stomach and bladder will be warm" (Berger, 1999). Another example is about female infertility. "A woman whose uterus is too cold within and too weak to conceive offspring can, if God wills, be aided to be fertile in the following way: take the uterus of either a lamb or a cow that is sexually mature but still pure in that it is not and has not been with young. Cook it with lard, other fatty meat and fat, and give that to the woman to eat when she has or will very soon have intercourse with her husband" (Berger, 1999).

As we compare the treatments from traditional West and East, both are associated with the imagination of the human body and medicinal materials. Before orthodox medicine revealed the real cause of disease, human beings had a long history of creative imagination about the inner and outer self. While ancient Chinese perceived the body in an adequate balance characterized by Yin and Yang, medieval European doctors treated a patient's coldness with the wine heated by fire. Since the hot/cold dichotomy is quite widespread in folk medicine, the principles of treatments are unsurprisingly the same (Foster, 1978). For example, similar to the way that the man-shaped ginseng is used to restore the inner energy of body, the uterus of either a lamb or a cow was also used for female infertility in medieval Europe.

DISCUSSION

Innovating Chinese Herbal Medicine

The 19th century witnessed the rise of modern medicine, nourished by science and technology during the same time. Since then, orthodox medicine became a scientific discipline supported by rigorous biomedical studies. However, it was until 20th century that TCM was reexamined through the lens of science. During the past decades, numerous studies were carried out by various methods in order to demystify the power of curative herbs (Normile, 2003; Tan and Vanitha, 2004; Jiang, 2005; Wang et al., 2006; Wang and Xiong, 2012).

Along with the persistent efforts of several generations of scientists, CHM indeed impressed the world with an effective therapy for people suffering from malaria. This drug is artemisinin, derived from *Artemisia annua* (Qinghao in Chinese), also known as sweet wormwood. During the 1960s, a group of scientists in China were assembled under a military project to find a treatment for malaria. Youyou Tu was one of them. After examining more than 2000 herbs, she narrowed down the targets to about 640. In 1971, Tu and her co-workers started to focus on herb Qinghao which showed promising inhibition to the parasite. However, this exciting observation couldn't repeat in the following experiments. Luckily enough, Tu found a medical classic by Ge Hong (284–346 CE). In *A Handbook of Prescriptions for Emergencies*, Ge wrote: "A handful

of Qinghao immersed with two liters of water, wring out the juice and drink it all." Different from a typical decoction of herbs, this unique preparation inspired Tu that heating would probably destroy the structure of the bioactive molecules. Soon, they modified the procedure to reduce the extraction temperature by using ethyl ether. The new sample of Qinghao ether extract showed 95–100% inhibition of rodent malaria. After this success, they began to isolate and purify the bioactive molecules in the extract. In 1972, an antimalarial compound was isolated with a formula of $C_{15}H_{22}O_5$, which was further crystalized and determined with the stereostructure in 1974. After the chemical structure was revealed, Tu and her co-workers studied its structure-efficacy correlation, which eventually led to the invention of dihydroartemisinin with improved efficacy (Tu, 2011, 2016). Artemisinin and its derivative dihydroartemisinin saved millions of lives threatened by malaria throughout the world. This discovery was considered one of the breakthroughs in human health during the last century. Due to her tremendous contributions, Tu was awarded the 2011 Lasker Award for clinical research and the 2015 Nobel Prize in Physiology or Medicine (Neill, 2011; Su and Miller, 2015).

The story of *A. annua* and Youyou Tu tells us the best outcome of herbal medicine in the context of science, that through the rigorous biomedical research, the healing mechanism of a herb was revealed from the ancient medical narratives. In other words, scientists are innovating CHM using scientific concepts and techniques, which provide us with a new understanding on this traditional health practice. Such techniques can be analytical chemistry (Jiang et al., 2010; Tistaert et al., 2011), systems biology (Wang et al., 2005, 2009), network pharmacology (Li et al., 2011; Li and Zhang, 2013), and computational modeling (Lukman et al., 2007; Barlow et al., 2012), with which, CHM is no longer what people perceived before World War II. It's approached with modern concepts, techniques, and methods, in the way that it can be appreciated by people all over the world.

Two Essential Questions from the Relationship of CHM and Science

When studying CHM, researchers usually argue if CHM is a science or can we apply scientific methods in the investigation. As a mixture of Chinese philosophy, culture, ritual and medical practices, CHM demands a comprehensive understanding which is not restricted to science. However, it doesn't prevent us from studying herbs by scientific methods and evaluating the performance of herbal medicine scientifically. In order to gain a contemporary understanding of CHM, we either seek the assistance of science or adopt a novel narrative approach (which will be discussed in the last section). Science bears its value in an unbiased standard that may fulfill CHM in the global setting just like artemisinin, which is no longer considered as herbal medicine but a universal antimalaria therapy acknowledged in and out of Chinese communities.

Another essential question: is it necessary for the scientific research of CHM to be guided by the records in medical classics? In the Chinese communities, we hear voices like the traditional narratives of herbs are still valid in the context of science; therefore, we need to make efforts to embody the ancient wisdom with scientific findings, as what Youyou Tu achieved in discovering artemisinin. While such an argument holds in limited scenarios, it's again not necessary to restrict science to these traditional writings. For example, although artemisinin was originally discovered as an antimalarial drug, recent studies show it also demonstrates anti-inflammatory, immunoregulatory, and anticancer functions which were not documented in any medical classics (Nakase et al., 2008; Shi et al., 2015). Neither do men put new wine into old bottles. Basically speaking, drug discovery in CHM is totally different from the knowledge in medical classics. In those classics, there is no modern concept of drug – molecules that can cure diseases. Furthermore, the concept of disease in the ancient Chinese context also bears a different notion far from what we understand in orthodox medicine. In the scientific study of the herbs, researchers can simply treat them as plants without any prior knowledge of healing potential because such narratives were not evaluated in any rigorous experiments and can be guiding and misleading at the same time. Therefore, to achieve an unbiased research of CHM, it's encouraged to put aside the medical classics although such information might offer some hints.

The Paradigm of Holism and Methodological Reductionism

Traditionally, a prescription of CHM is a unique formula tailored for the patient. In the formula, herbs are combined in a hierarchy of Principal, Associate, Assistant, and Coordinator. The herbs are also characterized according to their nature (hot, warm, cool, and cold) and flavor (acrid, sweet, bitter, sour, and salty), which need careful combination in the prescription (Chan, 1995). Doctors of TCM frequently advocate the philosophy of holism in medical practices as they hold firmly that everything is interconnected. Simply put, one's disease or syndrome is associated with various organs governed by the Five Elements theory. Therefore, the treatment as a prescription should also address different parts of the body. For example, a famous formula for the common cold is composed of four herbs: *Coptis chinensis*, *Scutellaria baicalensis*, *Phellodendron amurense*, and *Gardenia florida* (documented by Wang Dao), while the first three are responsible for clearing the heat at heart, lung, and kidney, respectively (Zeng et al., 2011).

Following the traditional practices of herbal medicine, some scientists adopt the paradigm of holism in drug discovery of CHM, as they believe this paradigm can best appreciate the ancient wisdom of herb combination (Leung et al., 2014). It's natural that if we stick to the traditional narratives of CHM, the formula should be evaluated as a whole and any separation inevitably results in decrease or even void in its performance. Ideologically speaking, holism distinguishes CHM as a unique health practice. However, while emphasizing the narrative on wholeness of herb and body, one is likely to neglect the building components of herbal medicine and their detailed mechanisms with drug targets.

Based on our standpoints on the two essential questions addressed above, we suggest methodological reductionism to be adopted in drug discovery of CHM. In order to acknowledge the

holistic nature of herb formula, it's a prerequisite to study the building blocks for understanding the complex system. To be concrete, here we would like to propose a three-step roadmap. First, it's fundamental to know the molecular elements in herbal medicine. For each herb, scientists are encouraged to identify as many molecules as possible and organize the information in databases. Secondly, we are obliged to clarify the toxicity and bioactivity of the identified molecules as it lays the foundation for future pharmaceutical study of the natural products. These two steps are also understood as reverse pharmacognosy for accelerating natural drug discovery (Do and Bernard, 2004). Finally, in order to reveal the mechanisms of a herb or formula, one can test the combinatorial function of composing compounds in the biological networks with respect to human diseases (Gu et al., 2013a,b). With that, the holistic practices in CHM can be revealed at the systemic level and engineered in a scientific manner. After a comprehensive understanding being achieved, we can further apply engineering approaches like systems biology to design tailored formulas targeting specific biological networks or diseases.

The Contemporary Narrative of Chinese Herbal Medicine in the 21st Century

However, as a double-edged sword, science also threatens CHM while fulfilling it because by the rigid scientific standards, the medical narratives of herbs are too vulnerable. Scientists and physicians, after rigorous examination, may discover an abundance of controversies between the facts and the classical writings. Especially during the past decades, CHM has been severely challenged on its efficacy and safety (Qiu, 2007). As a result, how can we appreciate herbal medicine in tandem with orthodox medicine? If we narrate CHM in the postmodern world full of scientific quests and proofs, what else can we offer other than the medical classics and scientific research? It is actually a very difficult question to answer; yet it is vital for the next generation of Chinese to keep their medical traditions.

As we have learned from our history, narrative is powerful because it keeps the identity of an ethnic group, transcending the collective memories into belief. Therefore, the questions on efficacy and safety raised by scientific research no longer hold back the faith in herbal medicine because CHM should not be judged only by facts, but also be appreciated by either individual or collective memories of the patients. A good example is the placebo effect (Kaptchuk, 2002). In order to construct the contemporary narratives of CHM, we suggest to alter the narrative subject from physicians to patients. Different from the orthodox medicine, CHM has a long history of practices in the homology of medicine and food. Herbs are never pure medicine in the Chinese context – they can be tonic or simple food. This special tradition lays the foundation for consumers to be the narrative subject, although their voices were neglected previously. Throughout the history, medical knowledges is usually documented or inherited by doctors, while the narratives of patients are undervalued (Charon, 2001). In the postmodern time, we hope to include patients as the narrator. Platforms like a forum could be a choice where patients can write to share their personal experiences of taking certain herbs in the treatment of certain diseases. With such a platform, people share their own clinical information of CHM, which can further be evaluated by researchers. Patients, both as the subject of disease and medical receiver, are appropriate and able to undertake the role of narrator, offering his own assessment of this alternative medicine, if not orthodox medicine, which requires professional knowledge to articulate (Greenhalgh and Hurwitz, 1999). With that, the narratives of CHM can be more comprehensive and robust in the 21st century.

CONCLUSION

In most current studies of Chinese herbal medicine, researchers examine the efficacy and safety of herbs from a scientific perspective. However, given the importance of its cultural and religious essence, the treatments of CHM are largely associated with the traditional narratives. After World War II, a group of Chinese scientists began to study CHM using scientific methods and achieved tremendous success in the discovery of artemisinin. As a result, CHM was innovated in the form of scientific drug discovery. However, this journey is not easy as we need to overcome the old ideology inherited from the history of TCM. The holistic principles of CHM also raise many debates. By addressing the two essential questions in the relationship of CHM and science, we upheld the paradigm of methodological reductionism and further proposed a three-step roadmap of drug discovery in herbal medicine. Finally, besides the scientific perspective, we suggested the contemporary narratives of CHM be shifted from physician based to patient based for the purpose of preserving this medical tradition as well as the ethnic identity.

AUTHOR CONTRIBUTIONS

SG and JP conceived the study and wrote the manuscript together.

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