

Supplementary Material

Supplementary Table 1. Information on Registered Medicines with Biomarkers Indicated in the SmPC or EPAR, Without Proposed CDx.

| International non- proprietary name (INN) | EU medicine brand name | Biomarker | Purpose of biomarker (BM) | Specific test mentioned in SmPC or EPAR |
|--|---------------------------|--|--|---|
| allopurinol/lesinurad | Duzallo | HLA-B*5801 | Complementary diagnostic for safety reasons in certain ethnic populations, not required | HLA-B*5801 genotyping assay |
| autologous CD34+ cells encoding βA-T87Q-globin gene | Zynteglo | TDT β0/β0 genotype | BM part of routine clinical assessment | - |
| azacitidine | Azacitidine Celgene | Marrow blasts | Part of complete blood count, clinical diagnosis | - |
| burosumab | Crysvita | Radiographic evidence of bone disease | BM serves clinical diagnosis | - |
| cerliponase alfa | Brineura | TPP1 | BM serves clinical diagnosis | - |
| cladribine | Mavenclad | Lymphocyte count | BM serves routine safety assessment | - |
| darunavir/cobicistat/emtricita bine/tenofovir alafenamide | Symtuza | HIV-1 RNA copies and CD4+ | BM part of routine clinical assessment | - |
| dolutegravir/lamivudine | Dovato | - | Absence of documented or suspected resistance to the integrase inhibitor class and to lamivudine serves clinical diagnosis | - |
| dolutegravir/rilpivirine | Juluca | NNRTI-associated mutations | BM may serve to clinically confirm absence of resistance | - |
| doravirine | Pifeltro | NNRTI-associated mutations | BM may serve to clinically confirm absence of resistance | - |
| doravirine/lamivudine/ enofovir disoproxil | Delstrigo | NNRTI-associated mutations | BM may serve to clinically confirm absence of resistance | - |
| dupilumab | Dupixent | Blood eosinophils and/or raised FeNO | BM serves clinical diagnosis type 2 inflammation | - |
| emicizumab | Hemlibra | Factor VIII inhibitors | BM serves clinical diagnosis | - |
| ertugliflozin | Steglatro | GFR | BM part of routine safety assessment | - |
| ertugliflozin/metformin hydrochloride | Segluromet | GFR | BM part of routine safety assessment | - |
| ertugliflozin/sitagliptin | Steglujan | GFR | BM part of routine safety assessment | - |
| fluciclovine (¹⁸ F) | Axumin | PSA | In vivo diagnostic | - |

| gemtuzumab ozogamicin | Mylotarg | CD33-positive | Complementary diagnosis, BM serves clinical diagnosis | - |
|---|-----------|--|---|-------------------|
| glibenclamide | Amglidia | G6PD | Warning for G6PD carriers | - |
| ibalizumab | Trogarzo | HIV-1 RNA copies | BM part of routine clinical assessment | - |
| lutetium (¹⁷⁷ Lu) oxodotreotide | Lutathera | Somatostatin receptor | In vivo diagnostic | - |
| metformin hydrochloride/ saxagliptin/dapagliflozin | Qtrilmet | GFR | BM part of routine safety assessment | - |
| mexiletine | Namuscla | CYP2D6 | Complementary diagnosis, BM serves safety assessment | |
| niraparib | Zejula | BRCA mutation | BM not used for patient selection | - |
| ocrelizumab | Ocrevus | Imaging features characteristic of inflammatory activity | In vivo diagnosis | MRI |
| peramivir | Alpivab | H275Y mutation in Influenza A/H1N1 virus | Warning for reduced susceptibility | PCR |
| sofosbuvir/velpatasvir/ voxilaprevi | Vosevi | HCV genotype | BM part of routine clinical assessment | biochemical assay |

Abbreviations: BM: biomarker, BRC: breast cancer suppressor gene, CD33: cluster of differentiation 33, CYP2D6: cytochrome P450 isoenzyme 2D6, FeNO: fraction of exhaled nitric oxide, G6PD: 6-phosphate dehydrogenase, GFR: glomerular filtration rate, HCV: hepatitis C virus, HIV-1: human immunodeficiency virus-1, MRI: magnetic resonance imaging, NNRTI: non-nucleoside reverse transcriptase inhibitor, PCR: polymerase chain reaction, PSA: prostate-specific antigen, TDT: transfusion-dependent β-thalassemia, TPP1: tripeptidyl-peptidase 1



Supplementary Table 2. Discussion on CDx Found in the EPARs for Products Registered at the EMA in 2017–2019

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| Discussion of analytical performance | | | |
|---|--|--|--|
| Besponsa (inotuzumab ozogamicin) | 1) Leukaemic cells were defined as CD22 positive if the level of fluorescence associated with binding of the CD22 monoclonal antibody [mouse anti-human monoclonal antibody clone RFB-4] was greater than the threshold value typically set based on the fluorescent intensity of leukemic cells stained with an isotype- matched control antibody. <u>The validation report</u> and addendums regarding peripheral blood specimens and inter-laboratory qualification <u>were provided</u> . (1) | | |
| | 2) Initially samples were considered to be positive if ≥20% of leukaemic blasts expressed surface CD22. <u>The cut-off value was</u> <u>subsequently changed to >0% blasts</u> (Protocol Amendment 2, StudyB1931022) based on the finding that some subjects that failed screening in the Phase 1/2 study B1931010 tested CD22 negative locally (≥20% cut-off) but were CD22-positive based on Central laboratory testing. (1) | | |
| lmfinzi (durvalumab) | The immuno-assays for quantification of durvalumab and soluble PD-L1 in human serum samples were adequately validated. (2) | | |
| Rubraca (rucaparib) | There was a high positive agreement between local and central BRCA results. The Clinical Trial Assay (as determined by a local laboratory) and CDx <u>results appeared consistent</u> . (3) | | |
| Rydapt (midostaurin) (cross)validation | Test assay performance was regularly monitored (every 6 months) throughout the study at these participating central labs through the use of <u>cross-validation panel testing</u> . Laboratory failure resulted in suspension until CTA proficiency was demonstrated in the following round. (4) | | |
| Tecentriq (atezolizumab) validation | Of note, although the immunohistochemistry assay is optimized to measure PD-L1 expression on both tumor cells (TCs) and tumour- infiltrating lymphocytes (ICs), the prevalence of PD-L1 expression on TCs in urothelial carcinoma is low. Therefore the VENTANA PD-L1 (SP142) IHC assay was <u>not validated for intended use to</u> <u>measure PD-L1 expression on TCs in urothelial carcinoma</u> . (5) | | |
| Vitrakvi (larotrectinib) | These techniques [Next Generation Sequencing (NGS) and Fluorescence in situ hybridization (FISH)] <i>might give rise to false-</i> | | |

positive results as some NTRK gene rearrangements detected on DNA appear not to produce a fusion transcript. (6)

| Discussion of clinical performance | | | |
|------------------------------------|--|--|--|
| Alecensa (alectinib) | For a new ALKi to be used after failure on prior ALKi treatment it is clinically relevant to define whether resistance is related to secondary ALK mutations or other mechanisms underlying resistance; a priori activity e.g., in case of resistance due to activation of alternative signalling pathways bypassing ALK appears remote. Similarly, to what degree the secondary mutation affects the activity of the new compound is of importance. Such data are presently available to a very limited and non-informative extent. <u>The applicant is</u> <u>recommended to provide final analysis on secondary ALK</u> <u>mutation positive/negative samples and correlation with clinical</u> <u>outcome.</u> (7) | | |
| lmfinzi (durvalumab) | The study recruited all-comers with regard to PD-L1 expression, however, in the pre-defined subgroup analyses based on PD-L1 expression the applicant has applied a cut-off for PD-L1 expression at 25%. However, as discussed above, it is not entirely clear how and why the cut-off is at 25%. The Applicant has been asked to provide subgroup analyses in patients with <1%, >= 1%, 5% and 50%, and compare and discuss these results with the results based the on the 25% cut-off. (2) | | |
| Lorviqua (Iorlatinib) | The approach used for ALK mutation RP2D selection is endorsed. <u>However, other relevant ALK-resistance mutations have been</u> <u>identified</u> for ceritinib (L1152R and F1174C/V) or alectinib (I1171N/T/S) [Muller et al. Onco Targets Ther. 2017; 10: 4535–4541] against which lorlatinib has not been tested in vitro using a PK/PD modelling-based approach. (8) | | |
| Mektovi (binimetinib) | In addition, genomic analysis of baseline samples remaining after centralized BRAF testing would be informative <u>to assess whether</u> <u>there is a relationship between baseline mutations and efficacy</u> <u>outcomes</u> . (9) | | |
| Rxulti (brexipiprazol) | The necessity of a mandatory genotyping assay should be discussed by the applicant. (10) | | |
| Rydapt (midostaurin) | The overall design of the study was considered adequate. The target patient population (patients with ≥5% FLT3-mutated alleles), the comparator (standard induction and consolidation chemotherapy plus placebo), and the sequential dosing regimen of midostaurin were considered acceptable and have been previously agreed upon in an SAWP scientific advice (EMEA/H/SA/764/1/2006/PA/III). (4) | | |

| Talzenna (talazoparib) | A biomarker research program in blood and tumour, based on the EMBRACA study, is ongoing. The Applicant has initiated next generation-based DNA sequence analysis of tumour tissue samples collected from patients enrolled in the Phase 3 Study 673-301 (EMBRACA; C3441009). Results from this analysis will include BRCA1/2 tumour mutational status and the tumour mutational status of over 300 other genes. The applicant is recommended to <u>submit</u> <u>the biomarker report</u> containing the final results by 31 March 2020. (11) |
|--|---|
| Tecentriq (atezolizumab) | which is partly attributed to the fact that PD-L1 expression proved to be rather of prognostic than of predictive value in this data set. Finally, the applicant is recommended to provide a "biomarker analysis plan" with timelines and should submit the results of all ongoing and planned biomarker analyses post-approval. (5) |
| Vitrakvi (larotrectinib) | 1) [An] expert expressed concern for the <u>very low percentage of</u> <u>breast cancers having proven NTRK fusions</u> as "oncogenic driver". (6) |
| | 2) Several molecular tools are currently available for the detection of NTRK fusions in tumour specimens: Immunohistochemistry (IHC). this tool must be validated against a sufficient cohort of NTRK- positive and –negative cases. Data on sensitivity and specificity on all available essays should be made available. (6) |
| Xospata (gilteritinib) | Exploratory analysis based on <u>different FLT-3 internal tandem</u> <u>duplication (ITD) allelic ratio cut-off levels</u> have been provided, based on 335 patients that tested positive for FLT3–ITD before inclusion in the study 2215-CL-0301. (12) |
| Discussion of intercha | ngeability of assays (concordance testing and bridging studies) |
| Besponsa (inotuzumab ozogamicin) | 1) To assess <u>concordance between local and central testing</u> , patients were divided into groups (\geq 90%, \geq 70- $<$ 90%, $>$ 0- $<$ 70%, 0%))) based on the % of CD22+ leukaemic blasts in their baseline specimen. Similar results were seen for both ALL studies. There was good concordance (~99.6%) for evaluable subjects (252/253) between central and local testing for CD22-positivity > 0%. However , concordance was poor (~37%) between Central and Local laboratories with regards to the level of positivity (\geq 90%, \geq 70 - $<$ 90%, 90%, >0 - $<$ 70%, 0%)). In general, a higher percentage of blasts were CD22-positive when measured by central versus local laboratory testing, suggesting that the central laboratory test was more sensitive. |

| | There was reasonable concordance (87.7%) between bone marrow and peripheral blood by Central laboratory for assessment of the % blasts that were CD22 + at baseline based on 57 evaluable patients. |
|---------------------------|--|
| | "The various local tests used in the two ALL clinical studies to screen for CD22 at baseline were not as sensitive as the single central laboratory test." Of these 367 patients, only one (who subsequently exhibited 86% CD22 positivity by central laboratory test) had CD22- negative B-cell ALL by local lab testing (ie, CD22 positivity=0%), consistent with CD22 positivity being an inclusion criterion for these studies. <u>It is not possible to determine the true proportion of</u> <u>locally tested patients who were CD22-negative</u> , since local laboratory CD22 test results were not formally captured for patients who failed screening and not all those who were screened negative locally were sent for central review. (1) |
| | 2) <u>The validation report</u> and addendums regarding peripheral blood specimens and <u>inter-laboratory qualification</u> were provided. (1) |
| Rubraca (rucaparib) | 1) Central testing in the clinical studies was initially performed using the clinical trial assay (CTA) and subsequently <u>bridged</u> to the CDx. The CTA (as determined by a local laboratory) and CDx <u>results</u> <u>appeared consistent.</u> |
| | 2) Tumour specimens from 79 patients in the primary efficacy (PE) population were received for central BRCA mutation testing at Foundation Medicine Inc. There was a <u>high positive agreement</u> <u>between local and central BRCA results</u> . (3) |
| Talzenna (talazoparib) | There are methods available in a research setting, to test for BRCA1/2 locus-specific loss of heterozygosity; however, <u>the</u> <u>Scientific Advisory Group (SAG) could not confirm to what</u> <u>extent any particular test is well-established.</u> Furthermore, the relationship between loss of heterozygosity (LOH) and Homologous recombination deficiency (HRD) in germline mutation-associated and sporadic breast tumours is unclear, and mechanisms apart from LOH do operate in gBRCA-associated breast cancer as a mechanism for biallelic inactivation of BRCA1 and BRCA2, so <u>the clinical utility of</u> <u>such tests [HRD testing] over BRCA testing is not likely to be</u> <u>important in the context of treatment effect with PARP</u> <u>inhibition.</u> (11) |
| Vitrakvi (larotrectinib) | However, given the multitude of 5' partners involved in NTRK1/2/3 fusion genes, assays that allow for the detection of multiple variants in a single test, including NGS-based RNA and DNA approaches, have been widely used in large academic centres. <u>The adoption of</u> <u>these NGS-based methods seems to be the better option</u> <u>despite that testing can also be performed with</u> <u>immunohistochemistry followed by confirmatory NGS</u> . (6) |
| Discussion of testing s | tored- vs fresh patient samples |

| Rubraca (rucaparib) | Local BRCA testing of DNA extracted from blood or buccal samples. Archival formalin-fixed paraffin-embedded tumour tissue samples were <u>retrospectively requested</u> to confirm the presence of BRCA mutations utilizing the FMI [foundation Medicine, Inc] validated tests (CTA then CDx). (3) |
|--------------------------|--|
| Vitrakvi (larotrectinib) | 1) Liquid biopsies should be further investigated to address this challenge. These [liquid biopsies] should be performed in projects with a joint tumour biopsy <u>up-front</u> and ideally at cancer progression. For the rare indications listed above, such <u>studies</u> <u>should be conducted to refine the understanding on patient</u> <u>selection</u> . (6) |
| | 2) CHMP questions with SAG answers: During the initial discussion, one expert raised the possibility to have a <u>tissue independent</u> <u>approval for cancers with proven NTRK fusions</u> as oncogenic "drivers", provided that next generation sequencing (NGS) could exclude other alterations being significant drivers for tumour progression. However, <u>data are lacking to establish the efficacy</u> <u>of such possible strategy</u> . |
| | A main drawback of the studies presented was the lack of comprehensive sequencing (NGS) of relevant tumour tissue reflecting the tumour characteristics at the time of treatment with larotrectinib. Such studies are necessary to understand the role of NTRK fusions in the context of other disease characteristics to allow patient selection and further evidence-based development in other indications or across groups of tumour types. Although the burden associated with biopsies is well-recognised most patients understand its importance and are willing to undergo the procedure. (6) |

References Supplementary Table 2

1. Besponsa EPAR, Public Assessment Report, procedure no. EMEA/H/C/004119/0000. https://www.ema.europa.eu/en/documents/assessment-report/besponsa-epar-public-assessment-report_en.pdf. Accessed Jun 11, 2020.

2. Imfinzi EPAR, Public Assessment Report, procedure no. EMEA/H/C/004771/0000. https://www.ema.europa.eu/en/documents/assessment-report/imfinzi-epar-public-assessment-report_en.pdf. Accessed Jun 11, 2020.

3. Rubraca EPAR, Public Assessment Report, procedure no. EMEA/H/C/004272/0000. <u>https://www.ema.europa.eu/en/documents/assessment-report/rubraca-epar-public-assessment-report_en.pdf</u>. Accessed Jun 11, 2020.

4. Rydapt EPAR, Public Assessment Report, procedure no. EMEA/H/C/004095/0000. <u>https://www.ema.europa.eu/en/documents/assessment-report/rydapt-epar-public-assessment-report_en.pdf</u>. Accessed Jun 11, 2020. 5. Tecentriq EPAR, Public Assessment Report, procedure no. EMEA/H/C/004143/0000. <u>https://www.ema.europa.eu/en/documents/assessment-report/tecentriq-epar-public-assessment-report_en.pdf</u>. Accessed Jun 11, 2020.

6. Vitrakvi EPAR, Public Assessment Report, procedure no. EMEA/H/C/004919/0000. <u>https://www.ema.europa.eu/en/documents/assessment-report/vitrakvi-epar-public-assessment-report_en.pdf</u>. Accessed Jun 11, 2020.

7. Alecensa EPAR, Public Assessment Report, procedure no. EMEA/H/C/004164/0000. https://www.ema.europa.eu/en/documents/assessment-report/alecensa-epar-public-assessment-report_en.pdf. Accessed Jun 11, 2020.

8. Lorviqua EPAR, Public Assessment Report, procedure no. EMEA/H/C/004646/0000. https://www.ema.europa.eu/en/documents/assessment-report/lorviqua-epar-public-assessment-report_en.pdf. Accessed Jun 11, 2020.

9. Mektovi EPAR, Public Assessment Report, procedure no. EMEA/H/C/004579/0000. <u>https://www.ema.europa.eu/en/documents/assessment-report/mektovi-epar-public-assessment-report_en.pdf</u>. Accessed Jun 11, 2020.

10. Rxulti EPAR, Public Assessment Report, procedure no. EMEA/H/C/003841/0000. https://www.ema.europa.eu/en/documents/assessment-report/rxulti-epar-public-assessment-report_en.pdf. Accessed Jun 11, 2020.

11. Talzenna EPAR, Public Assessment Report, procedure no. EMEA/H/C/004674. <u>https://www.ema.europa.eu/en/documents/assessment-report/talzenna-epar-public-assessment-report_en.pdf</u>. Accessed Jun 11, 2020.

12. Xospata EPAR, Public Assessment Report, procedure no. EMEA/H/C/004752/0000. <u>https://www.ema.europa.eu/en/documents/assessment-report/xospata-epar-public-assessment-report_en.pdf</u>. Accessed Jun 11, 2020.



Supplementary Table 3. Information on Registered Medicines With Companion Diagnostics, With the Brand Names of the CDx That Were Used in the Clinical Trials

Information taken from the SmPC or EPAR

| International non-proprietary name (INN) | EU medicine brand name | Biomarker | CDx brand used in clinical studies, as indicated in the SmPC or EPAR |
|--|------------------------------|---------------------------|---|
| alectinib | Alecensa | ALK | Ventana anti-ALK (D5F3) (IHC) |
| brigatinib | Alunbrig | ALK | Vysis ® Break Apart FISH Probe Kit |
| | | | FoundationOne NGS platform |
| lorlatinib | Lorviqua | ALK | Vysis ® Break Apart FISH Probe Kit |
| | | | Ventana ALK (D5F3) CDx Assay) |
| binimetinib | Mektovi | BRAF V600 mutation | n.m. |
| encorafenib | Braftovi | BRAF V600 mutation | n.m. |
| rucaparib | Rubraca | BRCA1/2 mutations | n.m. |
| talazoparib | Talzenna | BRCA1/2 mutations | Myriad BRACAnalysis test (CLIA and QSR/CDx versions) |
| dacomitinib | Vizimpro | EGFR activating mutations | Qiagen therascreen EGFR Mutations Detection Kit |
| | | | AmoyDxTM EGFR Mutations Detection Kit |
| gilteritinib | Xospata | FLT3 mutation | LeukoStrat® CDx FLT3 Mutation Assay |
| midostaurin | Rydapt | FLT3 mutation | n.m. |
| atezolizumab | Tecentriq | PD-L1 | Ventana PD-L1 (SP142) Assay |
| durvalumab | Imfinzi | PD-L1 | Ventana PD-L1 (SP263) IHC assay |
| inotuzumab ozogamicin | Besponsa | CD22 | n.m. |
| larotrectinib | Vitrakvi | NTRK gene fusion | NGS testing methods were used on DNA (MSK-IMPACT, FoundationOne, OncoPlex), RNA (Archer FusionPlex, OmniSeq comprehensive, Thermo Fisher Oncomine Focus), on circulating tumor DNA (Guardant360) |

| abemaciclib | Verzenios | HER2 negative | Other, according to ASCO guidelines |
|---------------------------|-----------|--|-------------------------------------|
| neratinib | Nerlynx | HER2 positive | PathVysion HER2 Kit (FISH) |
| ribociclib | Kisqali | HER2 negative | n.m. |
| brexpiprazole | Rxulti | CYP2D6 PM | n.m. |
| voretigene neparvovec | Luxturna | RPE65 mutations | n.m. |
| tezacaftor / ivacaftor | Symkevi | Homozygous for the F508del mutation or heterozygous + one of the mutations mentioned in the SmPC | n.m. |

Abbreviations: ALK: anaplastic lymphoma kinase, BRAF: B-raf proto-oncogene, BRCA: breast cancer gene, CD22: cluster of differentiation-22, CYP2D6: cytochrome P450 isoenzyme 2D6, EGFR: epidermal growth factor receptor, FISH: fluorescence in situ hybridization, FLT3: fms-like tyrosine kinase 3, HER: human epidermal growth factor receptor, IHC: immunohistochemistry, NGS: next generation sequencing, n.m.: not mentioned, NTRK: neurotrophic tyrosine kinase receptor, PD-L1: programmed death-ligand 1, RPE65: retinal pigment epithelium-specific 65 kDa protein