**SUPPLEMENTARY APPENDIX:**

**Distinct patters of Auto-Reactive Antibodies associated with organ specific Immune-Related Adverse Events**

# Contents

# Supplementary methods:

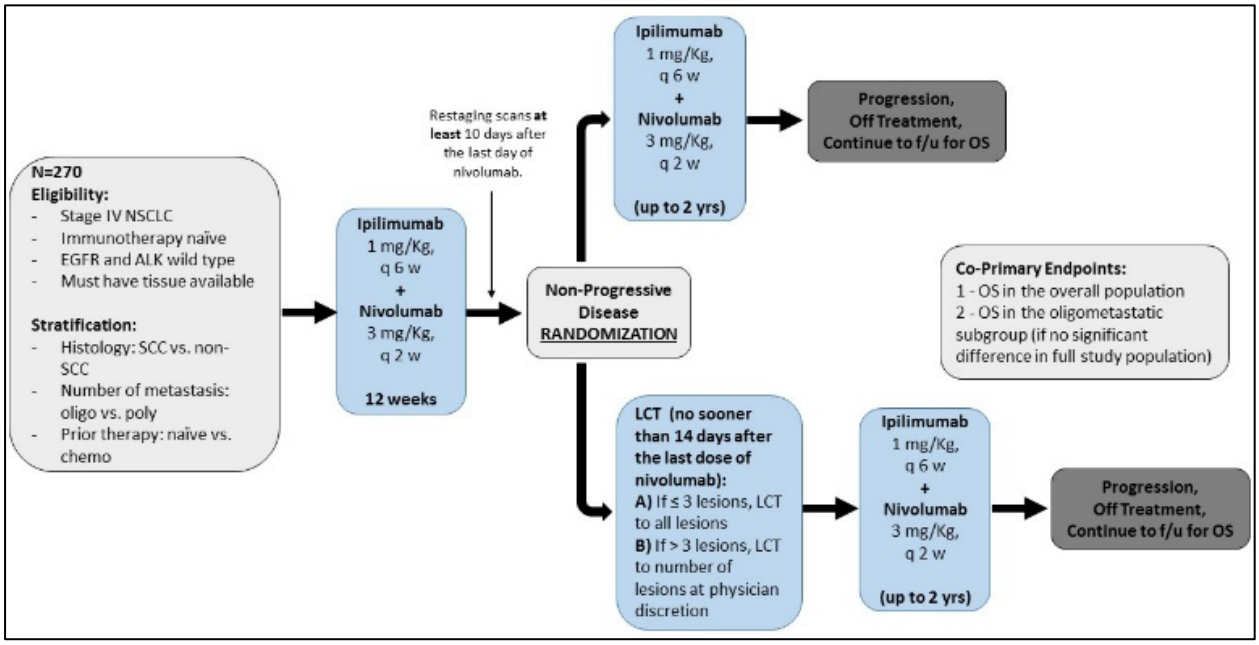
## 1) STUDY SYNOPSIS:

NCT 03391869 study synopsis.pdf

Major Inclusion Criteria: 1) Histologically confirmed NSCLC, 2) AJCC 8th edition stage IV disease, 3) EGFR wild type and no ALK fusion, 4) candidate for immunotherapy, and no prior history of this treatment; note that one prior course of chemotherapy is allowed, 5) candidate for radiation therapy to at least one site of disease.

Study Schema: Patients who do not progress after 12 weeks of treatment with ipilimumab/nivolumab will be randomized in a 1:1 fashion to: a) continuation of ipilimumab/nivolumab until progression or up to 2 years, vs. b) LCT, defined as surgery or radiation, with radiation to at least one site of disease plus ipilimumab/nivolumab until progression or up to 2 years. Surgery on the primary site will be strongly encouraged if feasible. For patients with oligometastatic disease, LCT will encompass all sites of disease. For patients with >3 lesions (polymetastatic disease), LCT will be performed on as many sites as the treating physician deems to be feasible and with acceptable tolerance (supplemental figure 1).

### Supplemental figure 1: Study schema




Statistical Considerations: Based on prior data from the Checkmate 017 and 057 (2, 3), as well as the PFS time for patients on first line systemic therapy, we estimated the OS time in the standard (ipilimumab/nivolumab) cohort to be 14.0 months. Based on data from our recently published

oligometastatic study, the OS time in this population was estimated to be 16.0 months. Sample size and effect size estimates are as follows:

A) OS assessment for the entire cohort (combined oligometastasis and polymetastasis).

• We plan to test the null hypothesis of no difference OS between patients randomized to LCT+ ipilimumab/nivolumab versus ipilimumab/nivolumab alone.

• Ho: $\rho = 1.0$ versus Ha: $\rho < 0.68$; where $\rho$ represents the hazard ratio (HR).

• A HR = 0.68, corresponding to a median OS benefit from 14 months to 20.6 months.

• A sample size of 216 randomized patients (108 patients per study arm) will yield approximately 80% power to detect a HR = 0.68 using a 1-sided log-rank test with a significance level of 0.05. This aspect of study requires a maximum of 178 events.

• The sample size is inflated (to account for 40% nonrandomization rate) to 360 patients due to potential dropouts from progression or toxicity during front-line immunotherapy (Or similarly, if approximately 40% of patients are expected to have a progression or toxicity during front-line immunotherapy; then 216/0.60 = 360 patients are required).

B) OS assessment for the oligometastatic cohort (to be done as primary endpoint if: a) 1A the null hypothesis (described above) is not rejected, or b) 1B as part of the futility analysis if stopping criterion for futility is met).

• We plan on randomizing a minimum of 74 patients with oligometastatic disease regardless of the outcome of the futility analysis, as noted above. Accruing a larger proportion of patients with oligometastatic disease during the study period may not be feasible given the number of patients that are seen at MD Anderson on a yearly basis. However, completing the enrollment will ensure that a sufficient number of patients are accrued to make the desired statistical comparisons between groups. A sample size of 74 patients with oligometastatic disease will provide 37 patients randomized to each study arm: LCT+ ipilimumab/nivolumab versus ipilimumab/nivolumab alone.

• A sample size of 74 patients yield 80% power with a 1-sided significance level of 0.05 to detect a HR = 0.51 using a 1-sided log-rank test with a significance level of 0.05.

• A HR = 0.51, corresponding to a median OS benefit from 16 months to 31.4 months and will require a maximum of 55 events.

Accrual Goal and Rate for the Study: The total patients to be accrued is 360 to ensure at least 216 patients are randomized (of which a minimum of 74 patients with oligometastatic disease will be acquired).

<u>Inclusion Criteria</u>

1) Age > 18

2) Histologically or cytologically confirmed non-small cell lung cancer. If a diagnostic biopsy is available, a pre-treatment biopsy is not required. Patients with a suspected lung cancer may be consented, but pathology must be confirmed prior to initiating treatment on study. Neuroendocrine carcinomas (e.g. SCLC, carcinoid tumors) are not eligible. Carcinomas with neuroendocrine differentiation are eligible.

3) Stage IV (according to AJCC 8th edition) measurable disease per RCESIST 1.1.

4) Signed and dated written informed consent prior to admission to the study in accordance with ICH-GCP guidelines and to the local legislation.

5) For lung adenocarcinoma patients, patients must not harbor any EGFR sensitizing mutations or ALK fusion where there are standard of care therapy options available. For patients with histologies other than adenocarcinoma, EGFR and ALK status is not required. Adenocarcinoma patients may be consented prior to the EGFR and ALK status being known, but EGFR and ALK status must be determined prior to initiating therapy. EGFR and ALK status may be determined using either tumor- or plasma-based, CLIA-certified assays. For patients with NSCLC, not otherwise specified (NOS), EGFR/ALK testing is not required, as the frequency of alterations is exceedingly rare in this histology. Also, note that patients with ROS1 or RET alterations can be enrolled, as Tyrosine inhibitor such crizotinib aren't established as first line therapy for patients with these alterations.

6) One prior line of chemotherapy and/or targeted agents for metastatic disease are permitted. This chemotherapy can include maintenance therapy, as long as it was given in the front line setting. In addition, prior antiangiogenic therapy (e.g. bevacizumab) is permitted if used as frontline treatment.

7) Patients must have organ and marrow function as defined below:

➢ Performance Status of 0 or 1 if using ECOG/Zubrod.

➢ Screening laboratory values must meet the following criteria and should be obtained within 14 days prior to treatment initiation

➢WBC≥ 2000/µL

➢Neutrophils≥ 1500/µL

➢ Platelets≥ 100 $x10^3$/µL

➢ Hemoglobin > 9.0 g/dL

➢ Serum creatinine ≤ 1.5 x ULN or creatinine clearance (CrCl) ≥ 50 mL (if using the Cockcroft-Gault formula below):

➢ Female CrCl = (140 - age in years) x weight in kg x 0.85 72 x serum creatinine in mg/dL

➢ Male CrCl = (140 - age in years) x weight in kg x 1.00 72 x serum creatinine in mg/dL

➢ AST/ALT ≤ 3 x ULN

➢ Total Bilirubin ≤ 1.5 x ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)

8) Women of childbearing potential (WOCBP) must use appropriate method(s) of contraception. Appropriate methods of contraception are as follows. Women will be instructed to adhere to contraception for a period of 26 weeks after the last dose of investigational product. Men receiving

nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 35 weeks after the last week of nivo/ipi.

Note: WOCBP is defined as any female who has experienced menarche and who has not yet undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition, women under the age of 55 must have a documented negative serum or urine test.

9) Women of childbearing potential must have a negative serum or urine pregnancy test within 48 hours prior to the start of nivolumab.

10) Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. Men receiving nivolumab and who are sexually

active with WOCBP will be instructed to adhere to contraception for a period of 35 weeks after the last dose of investigational product Women who are not of childbearing potential (ie, who are postmenopausal or surgically sterile as well as azoospermic men do not require contraception.

11) Subjects with brain metastases are eligible if metastases are adequately treated and subjects are neurologically stable (except for residual signs or symptoms related to the CNS treatment) for at least 2 weeks prior to the first dose of nivolumab. In addition, subjects must be either off corticosteroids, or on a stable or decreasing dose of ≤ 10 mg daily prednisone (or equivalent) for at least 2 weeks prior to the first dose of nivolumab. Patients with asymptomatic, small (e.g. ≤1 cm) brain metastases are eligible provided that the patient is off corticosteroids, or on a stable or decreasing dose of ≤ 10 mg daily prednisone (or equivalent) for at least 2 weeks prior to the first dose of nivolumab.

12) Subjects may receive radiotherapy for symptomatic metastases prior to enrollment provided that there is at least one other non-irradiated lesion amenable to LCT at the time of enrollment. When feasible, stereotactic body radiation therapy (SBRT) or other

hypofractionated techniques are strongly encouraged.

<u>Exclusion Criteria</u>

1) Systemic immunotherapy for metastatic NSCLC. Immunotherapy agents include, but are not limited to, agents targeting the PD1/PD-L1 axis (e.g. nivolumab, pembrolizumab, atezolizumab, durvalumab) or CTLA-4 (ipilimumab, tremelimumab) pathways.

2) Any approved anti-cancer therapy, including chemotherapy, or hormonal therapy within 3 weeks prior to the initiation of study treatment; the following exceptions are allowed:

o Hormone-replacement therapy or oral contraceptives

3) Women must not be breastfeeding.

4) Patients excluded with prior treatment of pneumonitis requiring corticosteroids within 60 days prior to the first dose of nivolumab.

5) Unwillingness or inability to follow the procedures required in the protocol.

6) Any serious or uncontrolled medical disorder that, in the opinion of the investigator, may increase the risk associated with study participation or study drug administration, impair the ability of the subject to receive protocol therapy, or interfere with the interpretation of study results.

7) Prior malignancy active within the previous 2 years. Patients with locally curable cancers that have been apparently cured, such as basal or squamous cell skin cancer, superficial bladder cancer, or carcinoma in situ of the prostate, cervix, or breast with local control measures (surgery, radiation) are eligible.

8) Patients should be excluded if they have an active, known or suspected autoimmune disease. Subjects are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger.

9) Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration (i.e. disease-modifying antirheumatic drugs). Inhaled or topical steroids and adrenal replacement doses > 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.

Note that subjects are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if >10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (e.g. contrast dye allergy) or for treatment of non-autoimmune conditions (e.g. delayed- type hypersensitivity reaction caused by contact allergen) is permitted.

10) As there is potential for hepatic toxicity with nivolumab or nivolumab/ipilimumab

combinations, drugs with a predisposition to hepatoxicity should be used with caution.

11) Patients should be excluded if they are known to be positive for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection.

12) Patients should be excluded if they have known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS).

13) History of allergy to study drug components.

14) History of severe hypersensitivity reaction to any monoclonal antibody.

15) Prisoners or subjects who are involuntarily incarcerated.

16) Subjects who are compulsorily detained for treatment of either a psychiatric or physical (infection disease) illness.

17) Psychological, familial, sociological or geographical factors potentially hampering compliance with the study protocol and follow-up schedule.

18) Any condition that, in the opinion of the investigator, would interfere with the study treatment or interpretation of the study results.

## 2) AUTOANTIGENS INCLUDED IN MICROARRAY:

All of the list of auto reactive antibodies, their description and vendor information is available in Supplemental table 2. Further information is also available at genomics and microarray core website https://genomics-microarray.swmed.edu and list of publications at https://genomics-microarray.swmed.edu/staff/publications/

### Supplementary Table 1: List of Auto reactive antibodies

| Auto antibody | Auto Antibody decription | *Vendor* |
|---|---|---|
| AChR3 | Acetylcholine receptor subunit gamma Recombinant Protein | MyBiosource.com |
| AFP | alpha-fetoprotein | Bio-Rad |
| ALDOC | Aldolase C | Genway |
| Aldolase (muscle) | Aldolase (muscle) | Sigma-Aldrich |
| α-2-macroglobulin-like 1 | Alpha-2-macroglobulin-like 1 | R&D Systems |
| Alpha-fodrin | Alpha-fodrin | cloud-clone corp. |
| AGTR1 | Angiotensin II Receptor Type 1 | MyBiosource.com |
| ANXA1 | Annexin A1 | Novoprotein |
| Apolipoprotein B/E | Apolipoprotein B and E | Sigma |
| Azurocidin | Azurocidin | Meridian Life Science, Inc. |
| BAFF | B-cell activating factor | Biolegend |
| Beta-glucuronidase | beta glucuronidase | Sigma |
| BRCA1 and BRCA2 | Breast Cancer gene 1 and 2 | abnova |
| CA125 | Cancer Antigen 125 | Fitzgerald |
| CA15-3 | Cancer Antigen 15-3 | LEE |
| CA19-9 | Cancer Antigen 19-9 | Meridian Life Science, Inc. |
| CA242 | Cancer Antigen 242 | Creative Biomart |
| CA27-29 | Cancer Antigen 27-29 | Fitzgerald |
| CA50 | Cancer Antigen 50 | LEE |
| CA72-4 | Cancer Antigen 72-4 | Fitzgerald |
| Calmodulin (brain) | Calmodulin (brain) | Calbiochem |
| Calreticulin(rabit calreticulin) | Calreticulin(rabit calreticulin) | Sigma Aldrich |
| Carbonic Anhydrase 6 (CA6) | Carbonic Anhydrase 6 (CA6) | R & D systems |
| Catalase | Catalase | Sigma |
| Cathepsin G | Cathepsin G | Enzyme Research Laboratories |
| CCL11 | C-C motif chemokine 11 | BioLegend |
| CCL2 | C-C motif chemokine 2 | BioLegend |
| CCL3 | C-C motif chemokine 3 | BioLegend |

| | | |
|---|---|---|
| CCL5 | C-C motif chemokine 5 | BioLegend |
| CDK2 | Cycline Dependent kinase 2 | Life Technology |
| CEA | Carcinoembryonic antigen | Fitzgerald |
| Cholinergic Receptor Muscarinic 3 (CHRM3) | cholinergic receptor muscarinic 3 | Abnova |
| CMV (G and M) | Cytomegalovirus Ig G and Ig M | Meridian Life Science, Inc. |
| CMV EXT (EXT-2??) | Cytomegalovirus | Meridian Life Science, Inc. |
| CMV GRADE III ANTIGEN | Cytomegalovirus Grade III Antigen | Meridian Life Science, Inc. |
| C-MYC | cellular-myc | Abcam |
| CTLA4 | cytotoxic T-lymphocyte–associated antigen 4 | Biolegend |
| CXCL10 | C-X-C motif chemokine ligand 10 | Biolegend |
| Cytokeratin 19 Ag | Cytokeratin 19 Ag | Biorad |
| DDX53 | DEAD-Box Helicase 53 | Abnova |
| dsDNA | Double stranded DNA | DIARECT |
| EBV EBNA1 | EBV/EBNA1 | Syd Labs |
| EGF Receptor | Epidermal growth factor receptor | Prospec-Tany-Technogene |
| Elastase | Elastase | Promega |
| Endothelial Cell Extract | Endothelial Cell Extract | Sciencellonline.com |
| Elastase | Elastase | Enzo life science |
| ErbB2 | Erb-B2 Receptor Tyrosine Kinase 2 | Biovision |
| ErbB3 and ErbB4 | Erb-B2 Receptor Tyrosine Kinase 3 and 4 | Biorbyt |
| ERP29 | Endoplasmic Reticulum Protein 29 | LSBio |
| Ferritin | Ferritin | Cell Sciences |
| Flagellin CBir1 | Anti-flagellin | Biorbyt |
| FUCA1 | Alpha-L-Fucosidase 1 | OriGene Technologies |
| Galactocerebroside | Galactocerebroside | MyBiosource.com |
| GBM | glomerular basement membrane | DIARECT |
| GBU4-5 (TDRD-12) | Tudor domain-containing 12 | Raybiotech |
| genomic DNA | genomic DNA | Roche |
| Glutamate decarboxylase-65/GAD2 | Glutamic acid decarboxylase | antibodies-online |
| Hepatitis A antigen | Hepatitis A antigen | Meridian Life Science, Inc. |
| HPV E6 (16) | Human papillomavirus E6 | Protein X Lab |
| HPV E7 (16+18) | Human papillomavirus E7 | Protein X Lab |
| HSV-1 and HSV-2 antigen | Herpes simplex virus 1 and 2 | Meridian Life Science, Inc. |
| HuD/ELAVL4 | ELAV-like protein 4 | Clonegene LLC |
| IFN α and β | Interferon alpha and beta | BioLegend |
| IFN-ɣ | Interferon gamma | BioLegend |
| IL-1 alpha and beta | Interleukin 1 alpha and beta | BioLegend |
| IL-12 (p70) | Interleukin-12 | BioLegend |
| IL-17A | Interleukin-17A | Biolegend |
| IL-2 | Interleukin-2 | Biolegend |

| | | |
|---|---|---|
| IL-6 | Interleukin-6 | Biolegend |
| IL-8 | Interleukin-8 | Biolegend |
| Lactoferrin | Lactoferrin | AbD Serotec |
| LC1 | Liver cytosol type 1 | DIARECT AG |
| LAMP-2 | Lysosomal Associated Protein 2 | DIARECT AG |
| MAG | Myelin-associated glycoprotein | LifeTech |
| MAGEA3 | Melanoma-associated antigen A 3 | Genway |
| MAGEA4 | Melanoma-associated antigen A 4 | Novus |
| MBP | Myelin basic protein (MBP) | EMD Millipore |
| MMP-2 | Metalloproteinase-2 | BioLegend |
| Muscarinic 3/CHRM3 | Cholinergic Receptor Muscarinic 3 | Abnova |
| NSE | Neuron-specific enolase | LEE |
| Nucleosome | Nucleosome | Arotec |
| NY-ESO-1 | New York Esophageal Squamous Cell Carcinoma-1 | Themo (Pierce) |
| OmpC | Outer membrane protein C | MyBiosource.com |
| Ox40L | Ox40L | BioLegend |
| P53 | Tumor protein 53 | Enzo life science |
| PSP | Parotid Secretory Protein | R&D Systems |
| PD-1 | Programmed cell death protein 1 | Abcam |
| PD-L1 | Programmed cell death protein-ligand 1 | Biorbyt |
| Peptidyl Arginine Deiminases 1-4 | Peptidyl Arginine Deiminases 1-4 | SignalChem |
| Phosphatidyl-l-serine | Phosphatidyl-l-serine | Sigma-Aldrich |
| PKM2 | Pyruvate kinase M 1/2 | Creative Biomart |
| PSA | Prostate Specific Antigen | Fitzgerald |
| PSMA | Prostate Specific Membrane Antigen | Fitzgerald |
| Prostatic Acid Phosphatase | Prostatic Acid Phosphatase | abcam |
| rhHSPG2 | Recombinant Heparan Sulfate Proteoglycan 2 | Abcam |
| Ribo Phospha Protein P0 P1 P2 | Ribo Phospha Protein P0, P1 and P2 | DIARECT |
| Ro-SSA (52 + 60) | Anti–Sjögren's-syndrome-related antigen A | DIARECT |
| Rotavirus SA-11 | Simian rotavirus | Meridian Life Science, Inc. |
| RSV antigen | Respiratory syncytial virus antigen | Meridian Life Science, Inc |
| RSVP antigen | Rubella spike viral protein antigen | Meridian Life Science, Inc |
| Rubella virus grade III and IV antigen | Rubella virus grade III and IV antigen | Meridian Life Science, Inc |
| Rubeola antigen | Rubeola antigen | Meridian Life Science, Inc |
| Saccharomyces Cerevisiae | Saccharomyces Cerevisiae | R & D systems |
| SCCA | Squamous cell carcinoma antigen | Creative Biomart |
| Sm/RNP | Sm/RNP | DIARECT |
| SOX2 | SRY-box 2 | Primorigen |
| Sphingomyelin | Sphingomyelin | Sigma-Aldrich |
| Tetanus toxoid | Tetanus toxoid | Sigma-Aldrich |

| | | |
|---|---|---|
| TGF-β1 | Transforming growth factor- beta 1 | Biolegend |
| TPO | Thrombopoietin TPO | DIARECT AG |
| Thyroglobulin | Thyroglobulin | DIARECT AG |
| TTG | Tissue Transglutaminase | DIARECT AG |
| TNF-α and β | Tumor necrosis factor-alpha and beta | Biolegend |
| Toxoplasma | Toxoplasma | Meridian Life Science |
| Troponin I | Troponin I | Meridian Life Science |
| Troponin I-T-C ternary complex mixture | Troponin I-T-C ternary complex mixture | Meridian Life Science, Inc |
| TWEAK (CD255) | TNF-like weak inducer of apoptosis | Biolegend |
| uPA | u-Plasminogen Activator | Abcam |
| VEGF-165 | Vascular endothelial growth factor 165 protein | BioLegend |
| VZV antigen | Varicella-zoster virus | Meridian Life Science, Inc. |

**Autoantibody controls**

Human IgG control
Human IgM control
anti-human IgG control
anti-human IgM control

## 3) BATCH CORRECTION FORMULA:

After the log2 transformation, the mean of the feature $j$ across all baseline samples within batch $k$ were calculated as shown in table 1 A, where $n_k$ is the number of baseline/control samples in batch $k$. Then, the batch corrected value $x^*_{ijk}$ for all samples was adjusted by where $N_k$ is the number of total samples in batch $k$. Modified from the mean centering batch correction method[1], shown in table 2 B.

### Supplemental table 2 A and B

| | |
|---|---|
| Table 2 A | $\bar{x}_{jk} = \frac{1}{n_k}\sum_{c=1}^{n_k} x_{cjk},$ |

| | |
|---|---|
| Table 2 B | $x^*_{ijk} = x_{ijk} - \bar{x}_{jk}, i = 1, 2, \ldots, N_k,$ |

# 4) CLINICAL CHARACTERISTICS:

## Supplementary Table 3. Clinicopathologic features of patients with/out irAE

| Age | Sex | Smoking hx | Prior chemo (Y: yes/ N: no) | Histopath | irAE 1 | Grade | Time (days) from first dose of I+N to irAE1 | If any other irAE | irAE 2 | irAE 2 grade | timing of irAE (overlap/seperate) | time from first dose of I+N to irAE 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 70 | M | Former | N | Adeno | diarrhea/colitis | 3 | 267 | | | | | |
| 53 | F | Former | Y | Large Cell | diarrhea/colitis | 3 | 422 | | | | | |
| 60 | M | Former | Y | Adeno | pneumonitis | 2 | | | | | | |
| 63 | F | Former | N | SCC | No tox control | | | | | | | |
| 85 | M | Former | N | SCC | diarrhea/colitis | 2 | 21 | | | | | |
| 47 | M | Never | N | SCC | No tox control | | | | | | | |
| 72 | M | Former | N | Adeno | diarrhea/colitis | 2 | 22 | yes | nephritis | 2 | separate | 91 |
| 64 | M | Former | N | Adeno | dermatitis | 2 | 84 | yes | Arthritis/Arthralgia | 2 | separate | 172 |
| 59 | M | Never | N | NOS | No tox control | | | | | | | |
| 71 | M | Former | N | Adeno | pneumonitis | 2 | 126 | yes | Artritis/Arthralgia | 2 | separate | 215 |
| 78 | F | Former | N | SCC | pneumonitis | 3 | 381 | | | | | |
| 70 | M | Former | Y | Adeno | No tox control | | | | | | | |
| 66 | M | Current | N | SCC | dermatitis | 2 | 91 | | | | | |
| 45 | M | Never | N | Adeno | No tox control | | | | | | | |
| 64 | M | Former | Y | Adeno | No tox control | | | | | | | |
| 54 | F | Never | Y | Adeno | hepatitis | 2 | 118 | | | | | |
| 54 | F | Never | Y | Adeno | No tox control | | | | | | | |
| 76 | M | Former | N | Adeno | No tox control | | | | | | | |
| 62 | F | Former | Y | Adeno | dermatitis | 2 | | | | | | |
| 67 | M | Former | N | SCC | No tox control | | | | | | | |
| 69 | F | Former | N | Adeno | diarrhea/colitis | 3 | 28 | | | | | |
| 57 | F | Former | N | Adeno | No tox control | | | | | | | |
| 82 | M | Former | N | SCC | No tox control | | | | | | | |
| 53 | M | Former | N | Adeno | diarrhea/colitis | 3 | 75 | | | | | |
| 73 | M | Former | N | Adeno | No tox control | | | | | | | |
| 83 | M | Former | N | SCC | hepatitis | 2 | 161 | | | | | |
| 68 | F | Former | Y | SCC | diarrhea/colitis | 3 | 24 | yes | Arthritis/Arthralgia | 2 | separate | 182 |
| 71 | F | Former | N | SCC | diarrhea/colitis | 2 | | | | | | |
| 74 | M | Former | Y | Adeno | pneumonitis | 2 | | | | | | |
| 58 | F | Former | N | Adeno | pneumonitis | 2 | | | | | | |
| 70 | F | Current | N | SCC | diarrhea/colitis | 2 | 92 | | | | | |
| 80 | M | Former | N | Adeno | pneumonitis | 3 | | | | | | |
| 72 | F | Former | N | Adeno | diarrhea/colitis | 3 | 70 | yes | Arthritis/Arthralgia | 3 | separate | 203 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 54 | F | Current | Y | Adeno | diarrhea/colitis | 2 | 21 | yes | adrenal insufficiency | 3 | separate | 73 |
| 56 | M | Current | N | SCC | pneumonitis | 2 | 457 | | | | | |
| 62 | M | Current | Y | SCC | No tox control | | | | | | | |
| 85 | M | Former | N | SCC | dermatitis | 2 | 405 | | | | | |
| 69 | F | Former | N | NOS | dermatitis | 2 | 28 | | | | | |
| 59 | M | Former | N | Adeno | No tox control | | | | | | | |
| 63 | F | Former | N | Adeno | pneumonitis | 2 | 196 | | | | | |
| 59 | F | Never | N | Adeno | pneumonitis | 3 | 66 | | | | | |
| 75 | M | Former | N | Adeno | dermatitis | 2 | 82 | | | | | |
| 70 | F | Former | N | Adeno | dermatitis | 2 | 147 | | | | | |
| 65 | M | Former | N | Adeno | diarrhea/colitis | 2 | 77 | yes | hepatitis | 3 | separate | 122 |
| 84 | M | Never | N | Adeno | No tox control | | | | | | | |
| 61 | F | Current | N | SCC | dermatitis | 2 | 131 | | | | | |
| 64 | F | Current | N | SCC | dermatitis | 2 | 28 | yes | diarrhea/colitis | 2 | separate | 65 |
| 68 | F | Former | Y | Adeno | hepatitis | 2 | | | | | | |
| 59 | F | Former | Y | Adeno | diarrhea/colitis | 3 | 77 | | | | | |
| 79 | F | Never | N | NOS | dermatitis | 2 | 89 | | | | | |
| 59 | F | Former | N | Adeno | hepatitis | 3 | 56 | | | | | |
| 77 | F | Former | N | Adeno | dermatitis | 2 | 90 | | | | | |
| 66 | M | Never | N | Adeno | No tox control | | | | | | | |
| 47 | F | Current | N | Adeno | No tox control | | | | | | | |
| 75 | F | Former | N | NOS | pneumonitis | 2 | 84 | | | | | |
| 72 | F | Former | N | Adeno | hepatitis | 3 | 49 | | | | | |
| 83 | F | Former | N | Adeno | diarrhea/colitis | 2 | 63 | | | | | |
| 49 | F | Never | N | Adeno | dermatitis | 2 | 70 | | | | | |

Clinical characteristics of the patients, details of the irAE event summarized in supplemental table 3.

# 5) IG G AND M AUTOREACTIVE ANTIBODIES IN PATIENTS WITH PNEUMONITIS

## Supplementary Table 4. IgG autoreactive antibodies in patients with pneumonitis at the time of toxicity

| Elevated IgG auto reactive antibodies at baseline | Elevated IgG auto reactive antibodies both at baseline and pneumonitis | Elevated IgG auto reactive antibodies at the time of pneumonitis |
|---|---|---|
| Cytokeratin 19 Ag | IL 17 A | Ca125 |
| CA242 | | Angiotensin II type 1 receptor |
| CCL3 | | CMT ext |
| | | SOX2 |
| | | IL12 |
| | | CEA |
| | | Enolase |
| | | SSCA |
| | | Beta Glucuronidase |
| | | CMV G and M |

Left panel (yellow) is a list of elevated Ig G auto reactive antibodies only at baseline in patients who experienced pneumonitis during ICI therapy compared with baseline samples of patients with no irAE during therapy. Middle panel (blue) is a list of elevated Ig G auto reactive antibodies not only at baseline (in patients with pneumonitis compared with baseline samples of patients with no irAE) but also elevated during pneumonitis event (when compared with patients' samples who received 12 weeks of ICI and had no irAE). Right panel (green) is a list of elevated Ig G auto reactive antibodies only at the time of pneumonitis event compared with patients' samples who received 12 weeks of ICI and had no irAE)

## Supplementary table 5. List of IgM Auto reactive antibodies elevated at baseline and at the time of pneumonitis event

| Elevated IgM auto reactive antibodies at baseline | Elevated IgM auto reactive antibodies both at baseline and pneumonitis | Elevated IgM auto reactive antibodies at the time of pneumonitis |
|---|---|---|
| Angiotensin II type 1 receptor | ACHRG (cholinergic receptor nicotinic gamma subunit) | Alpha-fodrin |
| Apolipoprotein B/E | Alpha fetoprotein | BAFF |
| BRCA1 and BRCA2 | Aldolase (muscle) | Beta-glucuronidase |
| CA19-9 | Aldolase-C | CA27-29 |
| CCL5 | Alpha 2 macroglobulin-like 1 | CA50 |
| CDK2 | CA125 | CA242 |
| DDX53 | CA72-4 | Carbonic anhydrase 6 |
| Endothelial cell extract | CCL3 | CD255 |
| Glutamate decarboxylase-65/GAD2 | CMV (G and M) | Carcinoembryonic antigen |
| HPV E6 | CMV EXT | dsDNA |
| HSV 1 and HSV 2 antigen | CMV grade III antigen | Enolase |
| IL-17A | CXCL10 | ErbB3 and ErbB4 |
| NY-ESO1 | Cytokeratin 19 | FUCA 1 |
| Outer membrane protein-C (E.coli) O | EGF receptor | Galactocerebroside |
| Prostate-specific antigen | ErbB2 | GBM |
| rhHSPG2 | Ferritin | GBU-4-5/TDRD-12 |
| RSV antigen | HPV E7 (16+18) | HuD/ELAVL4 |
| Rubeola antigen | IFN alpha and beta | IL-6 |
| Troponin I | IFN-gamma | Lactoferrin |
| Vascular endothelial growth factor-16 | IL-1 alfa and beta | MAGE A4 |
| | IL-2 | PKM2 |
| | IL-8 | PSMA |
| | IL-12 | rhHSPG2 |
| | LC1 | Ro-SSA |
| | Lysosomal-associated protein 2 | Rotavirus SA-11 |
| | MAGE A3 | RSV antigen |
| | MBP | SCCA |
| | Matrix metalloproteinase-2 | SOX-2 |
| | Myelin-associated glycoprotein | VZV antigen |
| | NSE | |
| | OX40L | |
| | Peptidyl arginine deiminases 1-4 | |
| | PD-1 | |
| | Prostatic acid phosphatase | |
| | Prostate-specific membrane antigen | |
| | Ribosomal phosphatase protein P0 P1 P2 | |
| | *Rubella* virus grades III and IV | |
| | Tetanus toxoid | |
| | Thrombopoietin | |
| | Thyroglobulin | |
| | Tissue transglutaminase | |
| | Toxoplasma | |

Left panel (yellow) is a list of elevated Ig M auto reactive antibodies only at baseline in patients who experienced pneumonitis during ICI therapy compared with baseline samples of patients with no irAE during therapy. Middle panel (blue) is a list of elevated Ig M auto reactive antibodies not only at baseline (in patients with pneumonitis compared with baseline samples of patients with no irAE) but also elevated during pneumonitis event (when compared with patients' samples who received 12 weeks of ICI and had no irAE). Right panel (green) is a list of elevated Ig M auto reactive antibodies only at the time of pneumonitis event compared with patients' samples who received 12 weeks of ICI and had no irAE)

# 6) FALSE DISCOVERY AND P VALUES FOR AUTO REACTIVE ANTIBODIES AT BASELINE

Baseline auto reactive antibodies during pneumonitis, dermatitis, hepatitis and diarrhea/colitis compared the auto reactive antibody panel with non irAE group (baseline samples of patients who did not have any immune related adverse events during at least 6 months of follow up (control)). Each organ specific toxicity analyzed separately.

Only auto reactive antibodies with a of p-value less than 0.05 has been summarized in below tables. False discovery rate and Fold change also provided in the table 6.1.A. and 6.1.B. for identified antibodies.

## Supplementary tables 6.1.A. and 6.1.B. Supplementary tables for PNEUMONITIS, baseline auto reactive antibody panel comparison for pneumonitis vs. non-irAE group, Ig G and Ig M:

### Supplementary table 6. 1.A) Ig G

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| Cytokeratin 19 Ag | 0.004843 | 0.619958 | 3.903535 |
| IL-17A | 0.019349 | 0.98776 | 1.805318 |
| CA242 | 0.034922 | 0.98776 | 2.008778 |
| CCL3 | 0.042168 | 0.98776 | 2.364204 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 6.1.B) IgM

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| OmpC (E coli outer membrane porin) | 0.00047 | 0.057215 | 4.351483 |
| HSV-1 and HSV-2 antigen | 0.002661 | 0.057215 | 4.254834 |

| | | | |
|---|---|---|---|
| IL-2 | 0.002973 | 0.057215 | 3.452312 |
| Alpha-2-macroglobulin-like 1 | 0.003656 | 0.057215 | 2.939609 |
| IL-1 alpha and beta | 0.003969 | 0.057215 | 3.478609 |
| IFN-gamma | 0.004024 | 0.057215 | 2.913485 |
| BRCA1 and BRCA2 | 0.004426 | 0.057215 | 3.895652 |
| Ox40L | 0.005105 | 0.057215 | 2.644983 |
| IFN alpha and beta | 0.005569 | 0.057215 | 2.412754 |
| LC1 | 0.005701 | 0.057215 | 2.926783 |
| MMP-2 | 0.005891 | 0.057215 | 3.396391 |
| Toxoplasma | 0.00614 | 0.057215 | 2.189032 |
| MBP | 0.006152 | 0.057215 | 2.516902 |
| MAG | 0.006543 | 0.057215 | 4.269352 |
| Ferritin | 0.007261 | 0.057215 | 2.664499 |
| rhHSPG2 | 0.007684 | 0.057215 | 2.917175 |
| VEGF-165 | 0.007886 | 0.057215 | 3.131167 |
| HPV E7 (16+18) | 0.009332 | 0.057215 | 3.919762 |
| IL-8 | 0.00948 | 0.057215 | 2.616495 |
| Tissue Transglutaminase (TTG) | 0.009831 | 0.057215 | 2.139732 |
| NY-ESO-1 | 0.009836 | 0.057215 | 2.553587 |
| MAGEA3 | 0.010134 | 0.057215 | 2.40144 |
| CA72-4 | 0.010281 | 0.057215 | 3.286973 |
| Prostate Specific Antigen (PSA) | 0.012214 | 0.062108 | 2.532473 |
| Rubella virus grade III and IV antigen | 0.012827 | 0.062108 | 2.028573 |
| CCL3 | 0.012947 | 0.062108 | 1.986482 |
| Cytokeratin 19 Ag | 0.013101 | 0.062108 | 2.366583 |
| Troponin I | 0.01508 | 0.068939 | 2.03792 |
| PSMA | 0.017032 | 0.070354 | 1.845458 |
| ErbB2 | 0.017147 | 0.070354 | 2.796617 |
| EGF Receptor | 0.018002 | 0.070354 | 2.319584 |
| IL-12 (p70) | 0.018236 | 0.070354 | 2.883134 |
| CDK2 | 0.018948 | 0.070354 | 2.177329 |
| CMV EXT | 0.019539 | 0.070354 | 2.692344 |
| Aldolase (muscle) | 0.019889 | 0.070354 | 2.021209 |
| Ribo Phospha Protein P0 P1 P2 | 0.02038 | 0.070354 | 2.157969 |
| CCL5 | 0.020914 | 0.070354 | 2.848887 |
| Angiotensin II type 1 Receptor | 0.021774 | 0.070354 | 2.217693 |
| Rubeola antigen | 0.021971 | 0.070354 | 3.392269 |
| RSV antigen | 0.021985 | 0.070354 | 2.297203 |
| Cholinergic Receptor Muscarinic 3 (CHRM3) | 0.023085 | 0.072071 | 2.586711 |
| CMV (G and M) | 0.0276 | 0.084114 | 2.94248 |
| NSE | 0.030239 | 0.085841 | 2.446971 |
| HPV E6 (16) | 0.030372 | 0.085841 | 1.876991 |

| | | | |
|---|---|---|---|
| Peptidyl Arginine Deiminases 1-4 | 0.030682 | 0.085841 | 2.32694 |
| CMV GRADE III ANTIGEN | 0.030849 | 0.085841 | 1.911743 |
| IL-17A | 0.032734 | 0.087593 | 1.892197 |
| DDX53 | 0.033702 | 0.087593 | 1.78545 |
| AFP | 0.033925 | 0.087593 | 3.353368 |
| PD-1 | 0.034602 | 0.087593 | 2.735907 |
| Thrombopoietin (TPO) | 0.0349 | 0.087593 | 3.828593 |
| Apolipoprotein B/E | 0.035692 | 0.087857 | 2.196748 |
| Prostatic Acid Phosphatase | 0.039089 | 0.091653 | 1.910915 |
| ALDOC | 0.039392 | 0.091653 | 1.898625 |
| Glutamate decarboxylase-65/GAD2 | 0.039722 | 0.091653 | 2.214697 |
| Endothelial Cell Extract | 0.040098 | 0.091653 | 1.835706 |
| Thyroglobulin | 0.043333 | 0.097309 | 2.457709 |
| CA125 | 0.044222 | 0.097593 | 9.390235 |
| Tetanus toxoid | 0.046764 | 0.100224 | 1.91369 |
| Lysosomal Associated Protein 2 (LAMP-2) | 0.04698 | 0.100224 | 2.054589 |
| CXCL10 | 0.048231 | 0.101205 | 2.315798 |
| CA19-9 | 0.049492 | 0.102176 | 3.944197 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 6.2.A. and 6.2.B. Supplementary tables for DERMATITIS, baseline auto reactive antibody panel comparison for dermatitis vs. non-irAE group, Ig G and Ig M:

### Supplementary table 6.2.A. IgG

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| dsDNA | 0.038594 | 0.997145 | 1.868919 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 6.2.B. IgM

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| IFN alpha and beta | 0.018835 | 0.456247 | 1.600547 |
| IL-2 | 0.024212 | 0.456247 | 1.833213 |
| Calreticulin | 0.041953 | 0.456247 | 1.455002 |
| Troponin I-T-C ternary complex mixture | 0.043846 | 0.456247 | 2.005458 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 6.3.A. and 6.3.B. DIARRHEA/COLITIS, baseline auto reactive antibody panel comparison for diarrhea/colitis vs. non-irAE group, Ig G and Ig M:

### Supplementary tables 6.3.A. Ig G

| None |
|---|

### Supplementary tables 6.3.B. IgM

| Marker | P value | FDR | FC |
|---|---|---|---|
| Hepatitis A antigen | 0.008004 | 0.261828 | 2.325275 |
| Thrombopoietin (TPO) | 0.008191 | 0.261828 | 2.964102 |
| VEGF-165 | 0.012916 | 0.261828 | 2.001886 |
| ACHRG | 0.016753 | 0.261828 | 2.007051 |
| Rubeola antigen | 0.017204 | 0.261828 | 2.257629 |
| DDX53 | 0.021716 | 0.261828 | 1.597522 |
| RSVP antigen | 0.027321 | 0.261828 | 1.957825 |
| genomic DNA | 0.027475 | 0.261828 | 2.249969 |
| NSE | 0.028795 | 0.261828 | 1.992499 |
| NY-ESO-1 | 0.029413 | 0.261828 | 1.595 |
| BAFF | 0.033133 | 0.261828 | 1.701828 |
| Tetanus toxoid | 0.041259 | 0.261828 | 1.684679 |
| dsDNA | 0.041653 | 0.261828 | 2.510088 |
| Enolase | 0.0439 | 0.261828 | 1.64521 |
| Toxoplasma | 0.047064 | 0.261828 | 1.572502 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 6.4.A. and 6.4.B. HEPATITIS, baseline auto reactive antibody panel comparison for hepatitis vs. non-irAE group, Ig G and Ig M:

### Supplementary table 6.4.A. Ig G

| None |
|---|

### Supplementary table 6.4.B. Ig M

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| CDK2 | 0.007867 | 0.351345 | 2.86715 |
| IL-2 | 0.017962 | 0.351345 | 2.927375 |
| Troponin I-T-C ternary complex mixture | 0.018433 | 0.351345 | 3.915224 |
| HSV-1 and HSV-2 antigen | 0.029983 | 0.351345 | 3.446584 |
| Rubeola antigen | 0.033589 | 0.351345 | 3.499312 |

| | | | |
|---|---|---|---|
| Saccharomyces Cerevisiae | 0.03516 | 0.351345 | 2.460669 |
| PD-1 | 0.041641 | 0.351345 | 3.083865 |
| CA72-4 | 0.048822 | 0.351345 | 2.621628 |
| Enolase | 0.049918 | 0.351345 | 2.136144 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

# 7) FALSE DISCOVERY AND P VALUES FOR AUTO REACTIVE ANTIBODIES AT TOXICITY

Auto reactive antibody panel at pneumonitis, dermatitis, hepatitis and diarrhea/colitis compared to 12-week control blood from patients who did not have any immune related adverse events. Each organ specific toxicity analyzed separately.

Only auto reactive antibodies with a of p-value less than 0.05 have been summarized in below tables.
IRAE event

## Supplementary tables 7.1.A. and 7.1.B. Antibody panel comparison for pneumonitis, at the time of toxicity event vs. 12-week control blood from non-irAE group

### Supplementary table 7.1.A. IgG

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| CA125 | 0.001817 | 0.033222 | 2.946284 |
| CMV EXT | 0.011699 | 0.166389 | 4.66718 |
| Angiotensin II type 1 Receptor | 0.01311 | 0.167805 | 2.581111 |
| SOX2 | 0.022562 | 0.262538 | 3.723643 |
| CEA | 0.039089 | 0.324464 | 6.352035 |
| Beta-glucuronidase | 0.042953 | 0.324464 | 1.991825 |
| IL-12 (p70) | 0.04428 | 0.324464 | 1.635602 |
| Enolase | 0.046512 | 0.324464 | 2.305693 |
| SCCA | 0.047879 | 0.324464 | 1.53579 |
| CMV (G and M) | 0.048064 | 0.324464 | 3.838923 |
| IL-17A | 0.048163 | 0.324464 | 1.55723 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary table 7.1.B. Ig M

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| ACHRG | 0.000185 | 0.012082 | 2.900841 |
| NSE | 0.000416 | 0.012082 | 3.7469 |
| BAFF | 0.000416 | 0.012082 | 2.79236 |
| Ox40L | 0.000474 | 0.012082 | 2.872867 |
| SCCA | 0.000723 | 0.012082 | 2.797048 |
| IL-6 | 0.000738 | 0.012082 | 2.654305 |
| LC1 | 0.000872 | 0.012082 | 2.532841 |
| Beta-glucuronidase | 0.000936 | 0.012082 | 2.79634 |
| Cytokeratin 19 Ag | 0.000995 | 0.012082 | 3.49091 |
| Toxoplasma | 0.00111 | 0.012082 | 2.406376 |
| CA72-4 | 0.001457 | 0.012082 | 3.831821 |
| Prostatic Acid Phosphatase | 0.001515 | 0.012082 | 2.464917 |
| IL-12 (p70) | 0.001519 | 0.012082 | 2.985453 |
| CA27-29 | 0.001644 | 0.012082 | 2.664336 |
| VZV antigen | 0.001656 | 0.012082 | 2.460694 |
| RSV antigen | 0.001699 | 0.012082 | 3.239714 |
| CMV EXT | 0.002045 | 0.013321 | 3.127093 |
| CXCL10 | 0.002179 | 0.013321 | 2.861287 |
| CA242 | 0.002309 | 0.013321 | 2.9559 |
| IFN-gamma | 0.002389 | 0.013321 | 2.588334 |
| Tetanus toxoid | 0.002394 | 0.013321 | 2.334613 |
| IL-2 | 0.002498 | 0.013321 | 3.328424 |
| IL-8 | 0.002775 | 0.014206 | 2.74355 |
| SOX2 | 0.003021 | 0.014872 | 8.122063 |
| GBU4-5/TDRD-12 | 0.003723 | 0.017018 | 2.159324 |
| MBP | 0.004138 | 0.018265 | 2.35889 |
| dsDNA | 0.004366 | 0.018627 | 4.146749 |
| Tissue Transglutaminase (TTG) | 0.004652 | 0.018851 | 2.036144 |
| MAGEA3 | 0.004713 | 0.018851 | 2.308581 |
| PSMA | 0.004903 | 0.019016 | 1.98888 |
| Peptidyl Arginine Deiminases 1-4 | 0.005091 | 0.019166 | 2.789099 |
| Ro-SSA (52 + 60) | 0.00526 | 0.019235 | 2.40439 |
| CA50 | 0.005544 | 0.019288 | 2.431079 |
| Rotavirus SA-11 | 0.005819 | 0.019288 | 2.311478 |
| Lysosomal Associated Protein 2 (LAMP-2) | 0.006008 | 0.019288 | 2.142733 |
| rhHSPG2 | 0.006013 | 0.019288 | 2.354179 |
| EGF Receptor | 0.006095 | 0.019288 | 2.67919 |
| CMV (G and M) | 0.006178 | 0.019288 | 3.626738 |
| Ferritin | 0.006959 | 0.021207 | 2.379112 |

| | | | |
|---|---|---|---|
| MAGEA4 | 0.00957 | 0.027632 | 2.315599 |
| AFP | 0.0097 | 0.027632 | 4.676667 |
| Galactocerebroside | 0.009715 | 0.027632 | 2.5634 |
| Ribo Phospha Protein P0 P1 P2 | 0.010254 | 0.028331 | 2.244509 |
| CEA | 0.010403 | 0.028331 | 3.993001 |
| Lactoferrin | 0.013171 | 0.033981 | 2.164509 |
| ErbB2 | 0.013274 | 0.033981 | 2.599646 |
| IL-1 alpha and beta | 0.01398 | 0.035087 | 2.540818 |
| FUCA1 | 0.014676 | 0.036125 | 2.487159 |
| ALDOC | 0.01588 | 0.038351 | 2.36874 |
| ErbB3 and ErbB4 | 0.016414 | 0.038394 | 2.236071 |
| TWEAK (CD255) | 0.016497 | 0.038394 | 2.415317 |
| Thyroglobulin | 0.017033 | 0.038933 | 2.464532 |
| MAG | 0.020889 | 0.046909 | 2.727135 |
| Alpha-fodrin | 0.022722 | 0.050146 | 1.965483 |
| Rubella virus grade III and IV antigen | 0.023812 | 0.051659 | 1.806415 |
| PD-1 | 0.025594 | 0.054601 | 2.460554 |
| CMV GRADE III ANTIGEN | 0.0287 | 0.059171 | 2.004832 |
| Enolase | 0.028792 | 0.059171 | 1.744474 |
| MMP-2 | 0.029123 | 0.059171 | 2.446438 |
| Thrombopoietin (TPO) | 0.032751 | 0.065501 | 2.785395 |
| Alpha-2-macroglobulin-like 1 | 0.033373 | 0.06572 | 2.103491 |
| Cholinergic Receptor Muscarinic 3 (CHRM3) | 0.035743 | 0.068909 | 2.125234 |
| Prostate Specific Membrane Antigen | 0.03607 | 0.068909 | 1.696648 |
| HuD/ELAVL4 | 0.03662 | 0.068932 | 1.915403 |
| CCL3 | 0.037993 | 0.070479 | 1.665462 |
| Carbonic Anhydrase 6 (CA6) | 0.039482 | 0.072196 | 1.797295 |
| PKM2 | 0.042244 | 0.076158 | 1.863357 |
| Aldolase (muscle) | 0.045024 | 0.080042 | 1.732216 |
| CA125 | 0.046933 | 0.082294 | 9.874557 |
| GBM | 0.047861 | 0.082786 | 1.753366 |
| HPV E7 (16+18) | 0.048712 | 0.082945 | 2.259987 |
| IFN alpha and beta | 0.049249 | 0.082945 | 1.778036 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 7.2.A. and 7.2.B. Antibody panel comparison for dermatitis, at the time of toxicity event vs. 12 week control blood from non-irAE group

### Supplementary table 7.2.A. Ig G

| None |
|---|

### Supplementary table 7.2.B. IgM

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| Cytokeratin 19 Ag | 0.016652 | 0.856594 | 2.004623 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 7.3.A. and 7.3.B. Antibody panel comparison for diarrhea/colitis, at the time of toxicity event vs. 12-week control blood from non-irAE group

### Supplementary table 7.3.A.

| Ig G none |
|---|

### Supplementary table 7.3.B.

| Ig M none |
|---|

## Supplementary tables 7.4.A. and 7.4.B. Antibody panel comparison for hepatitis, at the time of toxicity event vs. 12-week control blood from non-irAE group

### Supplementary table 7.4.A. IgG

| Marker | Pvalue | FDR | FC |
|---|---|---|---|
| Thyroglobulin | 0.008275 | 0.433068 | 3.261201 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 7.4.B. IgM

| None |
|---|

# 8) LONGITUDINAL CHANGES FOR AUTO REACTIVE ANTIBODIES IN TOXICITY GROUP WITH FALSE DISCOVERY AND P VALUES

Longitudinal blood samples compared at baseline and at the time of toxicity for pneumonitis, dermatitis, hepatitis and diarrhea/colitis. Each organ specific toxicity analyzed separately. Only auto reactive antibodies with a of p-value less than 0.05 has been summarized in below tables. IRAE event

## Supplementary tables 8.1.A. and 8.1.B. Antibody panel comparison at baseline and at the time of toxicity for pneumonitis

### Supplementary table 8.1.A. IgG

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| CA15-3 | 1.391581 | 0.015709 | 0.67023 |
| PD-1 | 1.821185 | 0.041548 | 0.714324 |
| CMV GRADE III ANTIGEN | 2.076585 | 0.048752 | 0.714324 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 8.1.B. IgM

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| ACHRG | 2.440366 | 0.030185 | 0.482962 |
| Calmodulin (brain) | 1.761404 | 0.04212 | 0.599041 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 8.2.A. and 8.2.B. Antibody panel comparison at baseline and at the time of toxicity for dermatitis

### Supplementary table 8.2.A. IgG

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| PD-1 | 4.785258 | 0.000389 | 0.04985 |
| CTLA4 | 1.355146 | 0.011895 | 0.453018 |
| Cytokeratin 19 Ag | **1.820377** | 0.016868 | 0.453018 |
| CA242 | 1.405426 | 0.017328 | 0.453018 |
| Thyroglobulin | 1.487418 | 0.017696 | 0.453018 |
| CA50 | 1.188069 | 0.03063 | 0.653435 |
| PD-L1 | 1.377312 | 0.042776 | 0.782187 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 8.2.B. IgM

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| Cytokeratin 19 Ag | 2.278222 | 0.014622 | 0.935838 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 8.3.A. and 8.3.B. Antibody panel comparison at baseline and at the time of toxicity for diarrhea/colitis

### Supplementary table 8.3.A. IgG

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| PD-1 | 3.658693 | 1.03E-05 | 0.001318 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 8.3.B. Ig M

| None |
|---|

## Supplementary tables 8.4.A. and 8.4.B. Antibody panel comparison at baseline and at the time of toxicity for hepatitis

### Supplementary table 8.4.A. Ig G

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| TGF-beta1 | 1.436922 | 0.003074 | 0.393525 |
| Lactoferrin | 1.251526 | 0.026332 | 0.84264 |
| Thyroglobulin | 6.135744 | 0.036159 | 0.84264 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 8.4.B. Ig M

| None |
|---|

## Supplementary tables 8.5.A. and 8.5.B. Antibody panel comparison at baseline and at the end of induction for pneumonitis

### Supplementary table 8.5.A IgG

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| PD-1 | 7.851721131 | 0.000983718 | 0.125915845 |
| IFN-r | -1.29097163 | 0.005932673 | 0.379691067 |
| CA15-3 | 1.226290415 | 0.011162789 | 0.476278985 |

### Supplementary table 8.5.B. IgM

| None |
|---|

## Supplementary tables 8.6.A. and 8.6.B. Antibody panel comparison at baseline and at the end of induction for dermatitis

### Supplementary table 8.6.A IgG

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| PD-1 | 8.091653015 | 1.95E-05 | 0.002492495 |
| CA242 | 1.318440872 | 0.008534644 | 0.449927412 |
| CTLA4 | 1.271357611 | 0.010545174 | 0.449927412 |
| Thyroglobulin | 1.564046903 | 0.041630237 | 0.638077109 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 8.6.B. IgM

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| Cytokeratin 19 Ag | 2.253686034 | 0.003547101 | 0.395050598 |
| CA242 | 1.623225592 | 0.006172666 | 0.395050598 |
| HuD/ELAVL4 | 1.26791986 | 0.030035606 | 0.605413722 |
| BAFF | 1.400794242 | 0.03141111 | 0.605413722 |
| CDK2 | 1.349071943 | 0.03329702 | 0.605413722 |
| P53 | 1.304894672 | 0.047240058 | 0.605413722 |
| Angiotensin II type 1 Receptor | 1.627571224 | 0.049174049 | 0.605413722 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary tables 8.7.A. and 8.7.B. Antibody panel comparison at baseline and at the end of induction for diarrhea/colitis

### Supplementary table 8.7.A IgG

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| PD-1 | 6.467974045 | 6.38E-06 | 0.000817235 |
| Angiotensin II type 1 Receptor | 1.203557096 | 0.027484903 | 0.634778899 |
| Cytokeratin 19 Ag | 1.586422923 | 0.028519128 | 0.634778899 |
| CTLA4 | 1.220990696 | 0.033394471 | 0.634778899 |
| CA242 | 1.375355232 | 0.034714471 | 0.634778899 |
| RSV antigen | 1.132981458 | 0.043533362 | 0.69227931 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 8.7.B. IgM

| None |
|---|

## Supplementary tables 8.8.A. and 8.8.B. Antibody panel comparison at baseline and at the end of induction for hepatitis

### Supplementary table 8.8.A IgG

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| Prostatic Acid Phosphatase | 1.160323632 | 0.005435085 | 0.373331431 |
| TGF-beta1 | 1.720855941 | 0.005833304 | 0.373331431 |
| Thyroglobulin | 8.791603298 | 0.01042671 | 0.435367164 |
| PSMA | 1.182967405 | 0.015069681 | 0.435367164 |
| PD-1 | 4.441416902 | 0.01700653 | 0.435367164 |
| Beta-glucuronidase | 1.318528755 | 0.026115771 | 0.477545522 |
| GBU4-5/TDRD-12 | 1.157427577 | 0.045394 | 0.64560356 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

### Supplementary table 8.8.B. IgM

| None |
|---|

# 9) LONGITUDINAL CHANGES FOR AUTO REACTIVE ANTIBODIES IN NO TOXICITY GROUP WITH FALSE DISCOVERY AND P VALUES

Longitudinal blood samples compared at baseline and at 12 weeks in patients who did not have any immune related adverse events during at least 6 months of follow up (control). Each organ specific toxicity analyzed separately.

Only auto reactive antibodies with a of p-value less than 0.05 has been summarized in below tables.
IRAE event

## Supplementary tables 9.A. and 9.B. Antibody panel comparison at baseline and in 12 weeks in no irAE group

### Supplementary table 9.1.A. Ig G

| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| PD-1 | 5.820913 | 4.71E-12 | 6.03E-10 |
| Prostatic Acid Phosphatase | 1.177405 | 0.000203 | 0.010746 |
| MAGEA4 | 1.166348 | 0.000252 | 0.010746 |
| MAG | 1.207892 | 0.000529 | 0.016935 |
| CA50 | 1.183669 | 0.00133 | 0.034041 |
| TWEAK (CD255) | 1.200066 | 0.002287 | 0.040937 |
| Lactoferrin | 1.24327 | 0.002817 | 0.040937 |
| CA15-3 | 1.180149 | 0.002911 | 0.040937 |
| IL-12 (p70) | 1.207435 | 0.002998 | 0.040937 |
| Rubeola antigen | 1.145529 | 0.003198 | 0.040937 |
| AFP | 1.277094 | 0.004859 | 0.056545 |
| Enolase | 1.160605 | 0.006054 | 0.060466 |
| TNF-alpha and beta | 1.126731 | 0.006411 | 0.060466 |
| GBM | 1.159052 | 0.006613 | 0.060466 |
| Thyroglobulin | 1.338449 | 0.009626 | 0.082143 |
| BRCA1 and BRCA2 | 1.519862 | 0.010919 | 0.085492 |
| rhHSPG2 | 1.294204 | 0.011558 | 0.085492 |
| dsDNA | 1.186232 | 0.012022 | 0.085492 |
| SCCA | 1.138406 | 0.014085 | 0.085529 |
| ACHRG | 1.158607 | 0.014518 | 0.085529 |
| CTLA4 | 1.266339 | 0.014662 | 0.085529 |
| HPV E7 (16+18) | 1.085288 | 0.014825 | 0.085529 |
| EGF Receptor | 1.564175 | 0.015369 | 0.085529 |
| RSV antigen | 1.188584 | 0.01724 | 0.085955 |
| IL-2 | 1.300793 | 0.017431 | 0.085955 |

| | | | |
|---|---|---|---|
| Alpha-2-macroglobulin-like 1 | 1.15459 | 0.017637 | 0.085955 |
| genomic DNA | 1.405329 | 0.018748 | 0.085955 |
| LC1 | 1.140303 | 0.018803 | 0.085955 |
| IL-8 | 1.412874 | 0.019498 | 0.08606 |
| Catalase | 1.144312 | 0.022277 | 0.095048 |
| MBP | 1.426453 | 0.023413 | 0.095865 |
| MAGEA3 | 1.169542 | 0.025365 | 0.095865 |
| Tissue Transglutaminase (TTG) | 1.102844 | 0.025777 | 0.095865 |
| Aldolase (muscle) | 1.139692 | 0.027005 | 0.095865 |
| Rotavirus SA-11 | 1.111267 | 0.027645 | 0.095865 |
| FUCA1 | 1.128885 | 0.028095 | 0.095865 |
| Prostate Specific Antigen (PSA) | 1.236224 | 0.028382 | 0.095865 |
| IL-1 alpha and beta | 1.157112 | 0.02846 | 0.095865 |
| Alpha-fodrin | 1.130194 | 0.029342 | 0.096301 |
| PKM2 | 1.187719 | 0.032044 | 0.10254 |
| NY-ESO-1 | 1.162953 | 0.034323 | 0.107156 |
| Toxoplasma | 1.179745 | 0.037734 | 0.112485 |
| EBV EBNA1 | 1.1231 | 0.038842 | 0.112485 |
| IFN alpha and beta | 1.419886 | 0.039502 | 0.112485 |
| Ribo Phospha Protein P0 P1 P2 | 1.134937 | 0.039545 | 0.112485 |
| Ferritin | 1.155557 | 0.040465 | 0.112598 |
| VZV antigen | 1.109393 | 0.041802 | 0.113843 |
| Sm/RNP | 1.159125 | 0.043217 | 0.114807 |
| CA242 | 1.194378 | 0.04395 | 0.114807 |
| CA27-29 | 1.107259 | 0.046018 | 0.117806 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

## Supplementary table 9.1.B. Ig M

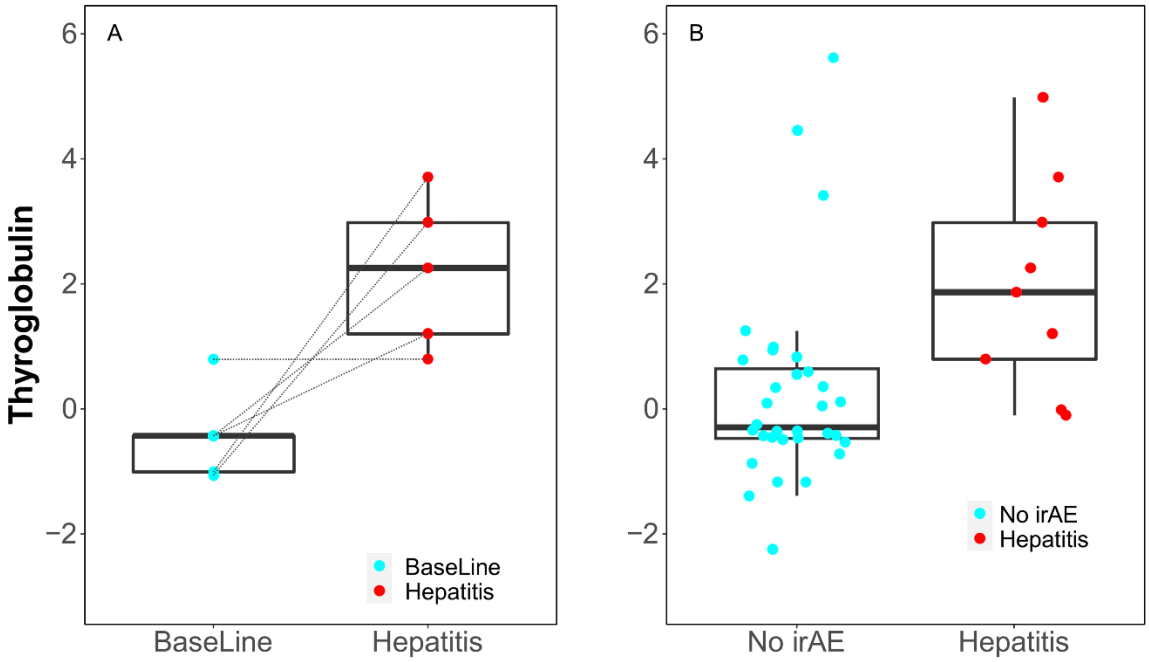
| Marker | FC | Pvalue | FDR |
|---|---|---|---|
| TWEAK (CD255) | 1.279557 | 0.000641 | 0.042997 |
| Enolase | 1.320217 | 0.000672 | 0.042997 |
| Aldolase (muscle) | 1.216801 | 0.001621 | 0.047378 |
| IL-12 (p70) | 1.425789 | 0.001853 | 0.047378 |
| MAGEA3 | 1.284817 | 0.001881 | 0.047378 |
| LC1 | 1.405077 | 0.002661 | 0.047378 |
| anti-Ig control 1:2 | 1.263456 | 0.002919 | 0.047378 |
| PD-1 | 1.482894 | 0.002961 | 0.047378 |
| Endothelial Cell Extract | 1.233854 | 0.00381 | 0.050206 |
| Ig control 1:8 | 1.148499 | 0.004239 | 0.050206 |

| | | | |
|---|---|---|---|
| rhHSPG2 | 1.494526 | 0.004746 | 0.050206 |
| Ig control 1:16 | 1.181706 | 0.005003 | 0.050206 |
| Tissue Transglutaminase (TTG) | 1.231777 | 0.00548 | 0.050206 |
| HPV E7 (16+18) | 1.525081 | 0.005491 | 0.050206 |
| ERP29 | 1.432012 | 0.006296 | 0.053728 |
| Rubella virus grade III and IV antigen | 1.273733 | 0.006748 | 0.053987 |
| CA50 | 1.151134 | 0.010611 | 0.079898 |
| Ig control 1:4 | 1.110331 | 0.014435 | 0.086258 |
| Ox40L | 1.255488 | 0.014833 | 0.086258 |
| OmpC | 1.430454 | 0.015947 | 0.086258 |
| Rubeola antigen | 1.713792 | 0.015977 | 0.086258 |
| Tetanus toxoid | 1.231265 | 0.016208 | 0.086258 |
| PD-L1 | 1.464174 | 0.016536 | 0.086258 |
| IFN-r | 1.360689 | 0.017086 | 0.086258 |
| Prostatic Acid Phosphatase | 1.215336 | 0.017932 | 0.086258 |
| dsDNA | 1.500508 | 0.018461 | 0.086258 |
| IFN alpha and beta | 1.281922 | 0.018643 | 0.086258 |
| CMV GRADE III ANTIGEN | 1.258457 | 0.018869 | 0.086258 |
| anti-Ig control 1:8 | 1.191849 | 0.023034 | 0.095468 |
| Ribo Phospha Protein P0 P1 P2 | 1.205245 | 0.024393 | 0.095468 |
| IL-1 alpha and beta | 1.385588 | 0.024613 | 0.095468 |
| Carbonic Anhydrase 6 (CA6) | 1.24751 | 0.025149 | 0.095468 |
| ANXA1 | 1.857097 | 0.025175 | 0.095468 |
| Saccharomyces Cerevisiae | 1.276888 | 0.025915 | 0.095468 |
| DDX53 | 1.194252 | 0.027823 | 0.095468 |
| Sphingomyelin | 1.23021 | 0.028206 | 0.095468 |
| VEGF-165 | 1.292925 | 0.02823 | 0.095468 |
| anti-Ig control 1:4 | 1.278613 | 0.028959 | 0.095468 |
| TNF-alpha and beta | 1.18627 | 0.029088 | 0.095468 |
| Prostate Specific Antigen (PSA) | 1.316446 | 0.03193 | 0.102177 |
| Alpha-2-macroglobulin-like 1 | 1.222932 | 0.033939 | 0.105163 |
| GBM | 1.137006 | 0.034507 | 0.105163 |
| CA72-4 | 1.332648 | 0.036384 | 0.108306 |
| GBU4-5/TDRD-12 | 1.141421 | 0.038494 | 0.111983 |
| AFP | 1.245907 | 0.046218 | 0.131465 |
| CCL3 | 1.18656 | 0.047353 | 0.131765 |
| Ig control 1:2 | 1.074154 | 0.049019 | 0.13265 |
| CMV EXT | 1.232647 | 0.049744 | 0.13265 |

P value significant if ≤0.05, FDR: False discovery rate, FC: Fold change

# 10) IgG ANTIBODY FOR THYROGLOBULIN IN HEPATITIS

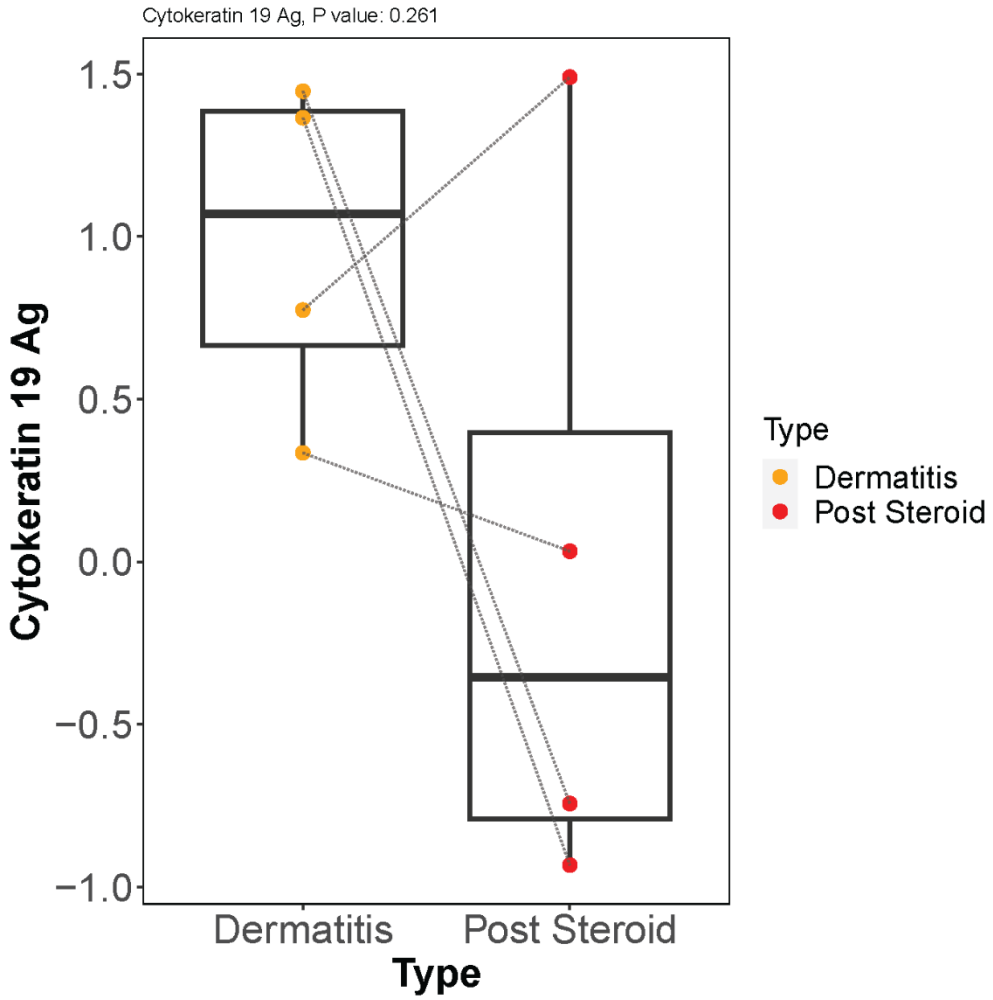
## Supplemental Figure 2. Box plots for IgG antibody for thyroglobulin




Supplemental figure 2: Box Plots of IgG antibody for Thyroglobulin. (A) Longitudinal serum samples from patients with hepatitis. Baseline: pre-I+N therapy, toxicity: at the time of hepatitis. (B) No irAE: Samples at 12 weeks of I+N therapy from patients with no irAEs. Hepatitis: at the time of grade ≥2 Hepatitis irAE .

## 11) AUTO REACTIVE ANTIBODY TITERS FOLLOWING STEROID USE:

### Supplemental figure 3. Longitudinal Cytokeratin 19 IgM titers in patients with dermatitis




Supplemental figure: Box plots of 4 patients who had G≥2 dermatitis ("dermatitis" samples at the time of toxicity) and treated with steroids ("Post steroid" samples are obtained after the systemic steroid use).

## REFERENCES


1. Sims AH, Smethurst GJ, Hey Y, et al. The removal of multiplicative, systematic bias allows integration of breast cancer gene expression datasets - improving meta-analysis and prediction of prognosis. *BMC Med Genomics.* 2008;1:42.