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A Randomized Phase II Trial of Nivolumab versus Nivolumab-Ipilimumab Combination in EGFR-Mutant Non-Small Cell Lung Cancer

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**Declaration of interests**
The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Dr Lai reported personal fees from Amgen and grants from Merck, Astra Zeneca, Pfizer, BMS and Roche outside the submitted work and sponsorship for meeting from DKSH. Dr A. Tan reported personal fees from Amgen, Pfizer and ThermoFischer Scientific, and sponsorship for meeting from Illumina. Dr Saw reported personal fees from Pfizer, Bayer, and sponsorship for meeting from MSD. Dr Gogna reports personal fees from Amgen. Dr Too reports research funding from NDR Medical outside the submitted work and personal fees from Sirtex Medical and Boston Scientific. Dr Kanesvaran reports personal fees from Merck, BMS and Novartis outside the submitted work. Dr Ng reports serving on advisory boards for Boehringer Ingelheim and Merck. Dr Ang reports personal fees from Boston Scientific and Pfizer, sponsorship for meeting from DKSH, Boehringer Ingelheim and Astra Zeneca and serving on advisory boards for Merck and Pfizer. Dr Toh reported serving on advisory...
boards for BMS. Dr Lim reports grants from BMS outside the submitted work, personal fees from MSD, Boehringer Ingelheim and Janssen and serving on advisory board for Novartis. Dr D.S.W. Tan reports grants from Astra Zeneca and Amgen, and personal fees from Novartis, Boehringer Ingelheim, Bayer, GlaxoSmithKline, Janssen, Amgen, and C4 Therapeutics outside the submitted work. No other disclosures were reported.

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Lung Cancer; Epidermal Growth Factor Receptor; Immunotherapy

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ABSTRACT

Background
While immune checkpoint inhibitors (ICI) have dramatically improved outcomes for non-oncogene addicted NSCLC, monotherapy with programmed death 1 (PD1) inhibition has been associated with low efficacy in the EGFR-mutant setting. Given the potential for synergism with combination checkpoint blockade, we designed a trial to test the activity of combination nivolumab-ipilimumab (NI) in EGFR-mutant NSCLC.

Methods
This is a randomized phase 2 study (NCT03091491) of nivolumab (N) versus NI combination in EGFR tyrosine kinase inhibitor (TKI)-resistant NSCLC, with crossover permitted on disease progression. The primary endpoint was objective response rate (ORR), and secondary endpoints included progression-free survival (PFS), overall survival (OS) and safety of ICI after EGFR TKI.

Results
Recruitment was ceased due to futility after 31 of 184 planned patients were treated. 15 received N and 16 received NI combination. 16 (51.6%) patients were PDL1≥1%, and 15 (45.2%) harboured EGFR T790M. 5 patients derived clinical benefit to ICI with 1 objective response (ORR 3.2%), and median PFS was 1.22 months (95% CI 1.15, 1.35) for the overall cohort. None of the 4 patients who crossed-over achieved salvage with NI. Programmed death ligand 1 (PDL1) and tumor mutational burden (TMB) were not able to predict for ICI response. Rates of all grade immune-related adverse events (irAE) were similar (80% vs 75%), with only two grade 3 events.

Conclusion
Immune checkpoint inhibition is ineffective in EGFR TKI-resistant NSCLC. While a small subgroup of EGFR-mutant NSCLC may be immunogenic and responsive to ICI, better biomarkers are needed to select appropriate patients.
INTRODUCTION

Immune checkpoint inhibitors (ICI) that abrogate the interaction of programmed cell death protein (PD-1) and programmed death-ligand 1 (PD-L1) have dramatically improved survival outcomes in patients with advanced non-small cell lung cancer (NSCLC)\(^1\text{–}^7\). Concomitant CTLA4 blockade has also been found to improve antitumor immunity through augmenting effector T-cell activation and reducing regulatory T cell dysfunction\(^8\), and has been shown to be a promising strategy to improve treatment outcomes in the metastatic setting\(^9\text{–}^11\) with a tolerable safety profile. Notably not all patients benefit from checkpoint blockade, and while several biomarkers that may predict for response to ICI have emerged such as PD-L1 expression levels\(^2,6,12\text{–}^14\), tumor mutational burden (TMB)\(^11,15,16\) and T cell-inflamed gene expression profile (GEP)\(^17,18\), the accuracy of these biomarkers are still unknown for many patient subsets.

Key to the treatment paradigm in the management of non-small cell lung cancer is the presence of oncogenic drivers such as the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) amongst others, where there is good response to tyrosine kinase inhibitors (TKI). Nonetheless acquired resistance to targeted therapies invariably develops, and strategies to overcome these mechanisms continue to be sought. While several trials\(^1,12,19\) evaluating immunotherapy in NSCLC allowed patients with EGFR and ALK mutations, only a minority of these patients were included across the studies, with generally low efficacy of ICI monotherapy regardless of PD-L1 status. Other studies specifically investigating the activity of PD-1 blockade in oncogene-addicted NSCLC have also reported poor efficacy\(^20\text{–}^22\). Further attempts at inducing long-term disease control of these patients have included combinatorial approaches with ICI and targeted therapy\(^23\text{–}^25\), which not only have not demonstrated synergistic or additive activity, but conversely have been found to result in increased grade 3 or higher toxicities. An increase in serious immune-related adverse events (irAE) has also been reported in patients treated sequentially with ICI followed by targeted therapy\(^26\), and thus the clinical implications of this approach must be carefully considered.
We sought to compare the efficacy and safety of nivolumab monotherapy against nivolumab-ipilimumab combination in patients with advanced EGFR-mutant NSCLC who have progressed on EGFR TKI.

MATERIALS AND METHODS

Patients

This is an open-label randomized phase 2 study done at National Cancer Centre Singapore. Eligible patients were aged 21 years and older, with either histologically or cytologically documented advanced (stage IIIB or IV according to seventh edition of TNM classification by American Joint Committee on Cancer, AJCC7) or recurrent NSCLC with a sensitizing EGFR mutation. Patients were required to have progressed on one line of standard EGFR TKI and not more than one line of chemotherapy. Patients with asymptomatic central nervous system (CNS) metastases were allowed provided they had had no ongoing requirement for corticosteroids as therapy for CNS disease, no stereotactic radiation with 7 days or whole-brain radiation within 14 days prior to randomization, and no evidence of interim progression between the completion of brain-directed therapy and screening radiographic study.

Key exclusion criteria included prior treatment with other anti-PD1, anti-PDL1 or anti-CTLA-4 therapies, active or previously documented autoimmune disease, or history of interstitial lung disease or pneumonitis. (Appendix 1: NCT03091491 Protocol)

Study design and treatment

Eligible patients were randomized (1:1) to receive intravenous nivolumab 3 mg/kg administered every 2 weeks (N), or nivolumab 3 mg/kg every 2 weeks and ipilimumab 1 mg/kg every 6 weeks (NI). Randomisation was stratified by PDL1 status (<1% vs ≥1%) and presence of brain metastasis. Patients and investigators were not blinded to treatment.
Treatment in both arms was continued until radiographic progression, unacceptable toxicity, investigator decision, or patient withdrawal of consent. Patients randomized to the N arm were allowed to crossover to the NI combination arm at time of progression. Patients were permitted to continue treatment beyond initial RECIST 1.1 defined progressive disease (PD) as long as they continued to derive clinical benefit as per investigator assessment, tolerated study drug(s), and did not have PD by immune-related RECIST (irRECIST).

**End points and assessments**

The primary end point for this trial was objective response rate (ORR, proportion of patients who experienced complete or partial response based on RECIST criteria). Secondary end points included progression free survival (PFS, time from randomization to date of first documented disease progression or death due to any cause, surviving patients who were free of progressive disease were censored at date of last documented tumour scan), overall survival (OS, time from randomization to the date of death due to any cause, surviving patients were censored at date of last follow up), duration of response (DoR), toxicity profiles of nivolumab with and without ipilimumab, as well as the salvage capability of the addition of ipilimumab to patients who progress on nivolumab alone to achieve clinical benefit.

Recruited patients were required to have a baseline imaging with computed tomography (CT) or magnetic resonance imaging (MRI) and tissue biopsy, followed by 6-weekly evaluation imaging, and thereafter every 12 weeks until disease progression. *EGFR* mutations were determined using Sanger sequencing at time of initial diagnosis. PD-L1 testing was done at point of randomization after exposure to *EGFR* TKI in the first-line setting. Adverse events and laboratory abnormalities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. An on-treatment tissue biopsy was required after 6 doses of treatment, and for patients progressing on nivolumab monotherapy prior to crossover to nivolumab-ipilimumab. Pre and post treatment biopsies were subject to whole-exome sequencing (WES) and total RNA sequencing, as previously described[27].
**Trial oversight**

The study protocol was conducted in accordance with Good Clinical Practice, as defined by the International Conference on Harmonisation (ICH). The study was approved by the institutional review board at each participating centre, and was conducted in compliance with the protocol. All patients provided written informed consent prior to enrolment. This trial was in part sponsored by Bristol Myers Squibb.

**Statistical analysis**

Given the published trial results\(^1,\)\(^9\) of Checkmate 057 and Checkmate 012, we aimed to investigate if the difference in ORR between patients treated with NI combination and N monotherapy was 20%, assuming 10% ORR in the N monotherapy group and 30% ORR in the NI combination group. With a 2-sided significance level of 5% and power of 90%, 184 patients (92 per treatment arm) were needed to compare the ORR of 10% versus 30% between the two treatments, based on the Chi-square test with Yates’ continuity correction.

Efficacy was analyzed on an Intention-To-Treat (ITT) basis. ORR for the two treatment arms was compared using the Chi-squared test. Logistic regression model, adjusting for the stratification factors, was performed to assess the robustness of the result. Patients with non-evaluable tumour response were treated as non-responders for the ORR primary analysis. Survival distribution for the secondary end points of DoR, PFS and OS was estimated using the Kaplan-Meier method. Median duration of follow up was estimated using the reverse Kaplan-Meier method. Safety analyses included all patients who received at least 1 dose of study treatment (as-treated population). All analyses were performed in SAS version 9.4 and R software version 3.6.3.

**RESULTS**

**Patients**
Thirty-one patients were enrolled on trial between April 2017 and December 2018. Three additional patients were screened but did not meet inclusion criteria (Figure 1). 15 patients were randomised to the N monotherapy arm, and 16 patients to the NI combination arm. Baseline clinical characteristics demonstrated that 19.3% of the entire cohort were smokers and ex-smokers, and nearly half (45.2%) were PDL1 negative (Table 1). Additionally, 45.2% harboured a EGFR T790M mutation, nearly all (13/14) of whom received prior third generation EGFR TKI. Of the overall cohort, 61.3% received prior third generation EGFR TKI prior to enrolment on trial. Approximately half (16/31) had brain metastases at baseline, 11 of whom (68.8%) had received prior brain-directed radiation therapy with stereotactic radiosurgery or whole brain radiation therapy. At the time of data cut-off, the median duration of follow-up was 24.3 months (IQR: 15.45, 24.33) and all patients had discontinued study treatment. The trial was terminated early in June 2019 due to clinical futility.

**Efficacy of immune checkpoint inhibition**

Amongst the 30 patients with evaluable disease, only one had an objective response to immunotherapy (ORR 3.2%, Figure 2A). This patient was randomized to the combination NI arm. There were no differences in PFS between the 2 arms, with a median PFS of 1.22 months (95% CI 1.15, 1.35) (Figure 2B) and a median overall survival of 5.65 months (95% CI 3.81, 10.59) for the overall cohort. 4 patients crossed over from the N monotherapy arm to the NI combination arm (patient numbers 4, 5, 9 and 20), but no salvage response was observed.

Five patients were found to have derived clinical benefit to immunotherapy, defined either by ongoing partial response (PR) or stable disease (SD) at 6 months, or a best response of PR (Figure 2C). All five patients were non-smokers and harboured an EGFR exon 19 deletion, with only one demonstrating a concomitant EGFR T790M mutation (Supplementary Table 1). In these patients, there was no evidence to suggest an association between PDL1 status and response to immune checkpoint inhibition, with only one patient with a PDL1 expression >50%. The median time to treatment failure on first-line EGFR TKI for these patients was 18 months (range, 9 – 76).
In terms of intracranial activity, 9 of the 16 patients (56.3%) with baseline brain metastases had intracranial progression. For the overall cohort, a total of 13 patients (41.9%) developed symptomatic brain metastases on study, 3 of whom had isolated intracranial progression.

**Safety**

Data regarding immune-related adverse events (irAE) are provided in Figure 3 and Supplementary Table 2. The rates of all grade irAE were similar in both arms (80% vs 75%). Dermatologic toxicity was the most frequently encountered irAE, followed by endocrine and gastrointestinal toxicities (Figure 3, Supplementary Table 2). One case of Grade 1 pneumonitis was observed in a patient in the N monotherapy arm. Two grade 3 or worse adverse events were observed in the overall cohort, despite the prior use of EGFR TKI immediately prior to trial entry for majority of the cohort (22/31). Both grade 3 toxicities were observed in the same patient (type 1 diabetes mellitus and myositis) from the NI combination arm. Amongst the four patients who crossed over from N to NI, there was generally no change in severity of irAE, with the exception of one patient who experienced a Grade 1 to 2 change in rash on crossover (Supplementary Figure 1).

**Effect of tumour microenvironment on response to immunotherapy**

To investigate tumour characteristics that may predict for response to immunotherapy, we identified 8 patients with target lesions which (1) either demonstrated shrinkage, or had a stable tumour size of 6 months or more; and (2) underwent tumour biopsy. We then performed detailed molecular analysis on these cases with adequate tumour tissue at baseline biopsy (n = 7; Supplementary Figure 2).

Exploratory analyses of TMB and GEP score\(^2^8\) were similar regardless of tumour response to immune checkpoint inhibition (Figure 4A-B). Other potential biomarkers of immunotherapy response such as CXCL9 expression\(^2^9\), CD39/CD73 pathways\(^3^0\), and the IDO pathway\(^3^1\) were also not associated with tumour response (Figure 4C).
To correlate treatment response with tumour inflammation status, a k-means clustering method of classification was applied to all baseline tumour transcriptome profiles (Supplementary Methods, RNA data analysis). This revealed that EGFR-mutated tumours were generally immune cold prior to immune checkpoint inhibition (n = 21/24) (Figure 5). However, this baseline inflammation state of the tumour was not predictive towards the eventual patient response to ICI therapy (Figure 5; \( P = 1 \); Fisher’s exact test). Similarly, using immune-cell gene expression signatures from Danaher et al.\textsuperscript{32}, we did not observe a correlation between inferred tumour immune-cell populations and treatment response (Supplementary Figure 3A).

To study the dynamics of treatment responses to immunotherapy, 8 paired baseline and on-treatment tumour samples were obtained from patients in the study. 4 of these paired samples were obtained from biopsies of tumour lesions with either shrinkage, or stability in size for 6 months of more (“biopsy-site responders”). Using a previously defined transcriptomic score for high immune infiltration (GEP\textsuperscript{hi} definition of \(-0.318^{38,50}\)), 2 of the 4 samples obtained from these biopsy-site responders were found to become GEP\textsuperscript{hi} while on treatment, while the third was already GEP\textsuperscript{hi} prior to treatment (Supplementary Figure 3B). In contrast, none of the tumour samples obtained from patients without a response to immunotherapy were classified as GEP\textsuperscript{hi} either before or during their course of treatment (Supplementary Figure 3B), suggesting that an immune-active tumor microenvironment, or the ability to engage an antitumor immune response, plays an essential role in patients with EGFR-mutant NSCLC during immunotherapy.

The inability of traditional biomarkers to predict for treatment response in our study prompted the search for novel biological mechanisms that could potentially account for the response to immune checkpoint inhibition in EGFR-mutant NSCLC. To this end, exploratory differential gene expression analysis was conducted, and RNA-seq data of baseline tumour samples from biopsy-site responders (n = 7) was compared against that of non-responders (n = 17).

Enrichment of several extracellular matrix (ECM) related genes such as \textit{CILP}, \textit{EFEMP1} and \textit{DPT}, as well as the upregulation of the epithelial-to-mesenchymal (EMT) promoting transcription factor PRRX1\textsuperscript{33}, was observed in tumour tissue obtained from
biopsy-site responders (Supplementary Figure 4A-C). Concurrently, gene set enrichment analysis (GSEA) also revealed the enrichment of matrisomal-related terms in the responders to immunotherapy at baseline (Supplementary Figure 4D), suggesting that the permissive effect of the extracellular matrix on the tumour microenvironment may be beneficial towards positive immunotherapy response within EGFR-mutant NSCLC.

**DISCUSSION**

To our knowledge, our study is the first published trial of combination PD-1/CTLA4 inhibition in patients with EGFR-mutant NSCLC. At the time of study initiation, Hellmann et al reported an objective response rate of 50% (4 out of 8) for EGFR-mutant NSCLC in Checkmate 012, providing rationale for combination checkpoint inhibition in this context. In this phase 2 randomized clinical trial, we established futility of immune checkpoint inhibition in the EGFR-mutant subgroup, which led to the decision for early termination of the study. Data from our cohort also demonstrates the inability of CTLA4 blockade to salvage failure of PD-1 inhibition, and adds to the existing evidence that immune checkpoint inhibition has poor efficacy in EGFR TKI-resistant NSCLC.

Genomic and transcriptomic profiling of the EGFR TKI-resistant tumour samples from our cohort revealed an immune-cold phenotype, which is in keeping with prior reports of an uninflamed tumour microenvironment with immunological tolerance and weak immunogenicity in the EGFR-mutant NSCLC context. This has been postulated to account for a poor response to immunotherapy, and identification of predictive biomarkers of immunotherapy response in context of EGFR-mutant NSCLC remains a challenge. While PD-L1 expression thresholds are now commonly used in clinical practice to select for patients with the highest likelihood of clinical response to immunotherapy, its role in this molecular subtype remains controversial, with conflicting reports regarding the correlation between EGFR mutation and PDL1 expression. The limitation of PDL1 as a biomarker in EGFR TKI-resistant NSCLC was also demonstrated in our study, and possibly reflects the impact that an immune suppressive TME comprising mainly of T-regulatory cells (Tregs), myeloid-derived
suppressor cells (MDSCs) and tumour-associated macrophages (TAMs) has on ICI resistance.

Given data from our prior work where we observed an association between an inflamed tumour microenvironment and TKI-resistant states, a further attempt was made to investigate baseline tumour immune phenotype and its impact on clinical response to ICI, but no association was demonstrated (Supplementary Figure 5). Transcriptomic analysis of the pre-ICI tumour samples in our cohort also determined that previously described biomarkers for immunotherapy response such as TMB, GEP score, CXCL9/13 expression, and CD39/CD73 or IDO pathway upregulation, were unable to predict for response in the EGFR TKI-resistant setting. Similarly, we did not observe significant overlap in genes associated with response in our study and a previously published cohort of anti-PD1/PD-L1 treated EGFR-wt NSCLC. Overall, these results led us to hypothesize that an immune-favourable ECM environment may potentially predict for positive immunotherapy response in the context of EGFR-mutated, TKI-resistant tumours, and thus plans to validate select ECM or TME-related genes are underway. Interestingly a T-cell inflammation profile switch from a GEPlo to GEPhi phenotype was observed in 2 (out of 4) of the biopsy-site responders, suggesting that EGFR-mutant NSCLC may still retain the ability to mount an anti-tumour response, and that other yet-to-be uncovered immune mechanisms are at play to influence T-cell responses and ICI efficacy.

The issue of central nervous system (CNS) control in the context of EGFR-mutant NSCLC is also an important point of consideration in the approach of patients who have progressed on targeted therapy. EGFR-mutated tumours have a known predilection for brain metastasis, with a baseline incidence of 20 to 30%, and a 5-20% risk of CNS progression while on EGFR TKI. In our study, over 40% of our overall cohort developed intracranial failure on ICI, highlighting the inadequacy of this approach in attaining intracranial control in oncogene-addicted cancers when CNS-penetrant TKIs are discontinued. While combination EGFR TKI and T-cell based therapy was previously investigated on the hypothesis of enhanced antitumor immunity through downregulation of PD-L1, the challenge of overcoming additive toxicities still remains, with potentially fatal consequences such as interstitial lung disease. To this end, novel therapeutic approaches which aim to more specifically
increase the immunogenicity of EGFR TKI-resistant tumour cells or to inhibit immunosuppressive signalling in the TME deserve further evaluation in this area of clinical unmet need.

In summary, in spite of the small cohort size, our study was able to demonstrate the futility of ICI in the EGFR TKI-resistant NSCLC setting. No new safety concerns were identified despite the sequential use of EGFR TKI followed by ICI in majority of patients. Though a small subgroup of EGFR-mutant lung cancer patients with a GEP\textsuperscript{hi} phenotype may potentially benefit from ICI, real time clinical application remains challenging, and in the absence of better biomarkers to select appropriate patients, PD-1/CTLA-4 inhibition alone is inadequate to confer clinical benefit in the treatment of EGFR TKI-resistant NSCLC.
REFERENCES


Table 1 Patient characteristics

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Assessed for eligibility (n = 34)

31 Randomized

15 Were assigned to receive nivolumab monotherapy (N)
15 Received assigned treatment

15 Discontinued treatment
12 Had progressive disease
2 Had adverse events
0 Withdrawed consent
1 Died

At data cutoff:
0 Continued to receive N
1 Were being followed for survival after discontinuation
0 Withdrawed consent
14 Died
0 Lost to follow up

15 Were included in the intention-to-treat analysis
15 Were included in the safety analysis

16 Were assigned to receive nivolumab + ipilimumab (NI)
16 Received assigned treatment

16 Discontinued treatment
13 Had progressive disease
1 Had adverse events
1 Withdrawed consent
1 Died

At data cutoff:
0 Continued to receive N
1 Were being followed for survival after discontinuation
0 Withdrawed consent
13 Died
1 Lost to follow up

16 Were included in the intention-to-treat analysis
16 Were included in the safety analysis

3 Excluded
(Did not meet inclusion criteria)
Fig. 2 Clinical outcomes for EGFR TKI-resistant patients on nivolumab with or without ipilimumab. 
A Waterfall plot for patients with evaluable radiographic images. B Progression-free survival of patients on nivolumab (N) and nivolumab-ipilimumab (NI). C Spider plot of individual tumor responses.

### Best Response for Target Lesions

<table>
<thead>
<tr>
<th></th>
<th>Nivolumab (%)</th>
<th>Nivolumab-Ipilimumab (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial response</td>
<td>0 (0.0)</td>
<td>1 (6.3)</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>6 (40.0)</td>
<td>6 (37.5)</td>
<td>12 (38.7)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>8 (53.3)</td>
<td>9 (56.3)</td>
<td>17 (54.8)</td>
</tr>
</tbody>
</table>

### Progression-Free Survival

<table>
<thead>
<tr>
<th></th>
<th>Median PFS in months (95% CI)</th>
<th>3-month PFS rate (95% CI)</th>
<th>6-month PFS rate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>1.31 (1.15, 2.40)</td>
<td>16.7% (2.9%, 40.2%)</td>
<td>8.3% (0.5%, 30.6%)</td>
</tr>
<tr>
<td>Nivolumab-Ipilimumab</td>
<td>1.18 (1.15, 3.88)</td>
<td>31.3% (11.4%, 53.6%)</td>
<td>12.5% (2.1%, 32.8%)</td>
</tr>
<tr>
<td>Overall</td>
<td>1.22 (1.15, 1.35)</td>
<td>25.1% (11.5%, 41.3%)</td>
<td>10.8% (2.8%, 24.9%)</td>
</tr>
</tbody>
</table>

### Individual Tumour Response

- **No clinical benefit**
- **Clinical benefit**
**Fig. 3** Immune-related adverse events (irAE) in patients on Nivolumab (N) and Nivolumab-Ipilimumab (NI)

- **Hepatic**
  - N: 1 (6.3%)
  - NI: 1 (6.3%)

- **MSK**
  - N: 1 (6.7%)
  - NI: 1 (6.3%)

- **Endocrine Pulmonary**
  - N: 2 (13.3%)
  - NI: 1 (6.3%)

- **GI**
  - N: 3 (18.8%)
  - NI: 1 (6.3)

- **Skin**
  - N: 5 (33.3%)
  - NI: 2 (12.5%)

MSK, musculoskeletal; GI, gastrointestinal
Fig. 4 Conventional biomarkers of immunotherapy response do not stratify for treatment responses in patients with EGFR mutant NSCLCs. A) T-cell inflammation scores (GEP score) of pre-ICI treated patient tumours. B) Tumour mutation burden (TMB) of pre-ICI treated patient tumours. C) Gene expression profiles (LogCPM) of several additional biomarkers of immunotherapy responses in pre-ICI treated patient tumours. Expression of CXCL13 was not detected from our RNA-seq and as such, data is not presented. Student’s T-test was used to determine the significance of observed expression differences between cohorts, with the corresponding p-value presented within the figure. Blue bars: ICI biopsy-site responders (Res), Red bars: ICI biopsy-site non-responders (Non-Res).
**Fig. 5** Tumour immune phenotype of EGFR-mutant NSCLC prior to receiving ICI. Tumours were classified into “immune-hot” or “immune-cold” status based on gene expression levels of the GEP genes with CTLA4, ENTPD1 and CD38 expression included (see Supplementary Methods). Genes that are upregulated are expressed as positive Z-scores (red), while genes that are downregulated are distinguished by negative Z-scores (blue).
CRediT Statement

1. Gillianne G.Y. Lai: Data curation; Formal analysis; Investigation; Methodology; Project administration; Visualization; Roles/Writing - original draft; Writing – review and editing.
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33. Daniel S.W. Tan: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing – review and editing.