Abstract O85 Table 1 Advanced anti-PD-1 Refractory melanoma patient population by treatment allocation

	CMP-001 + Pembrolizumab N = 144				CMP-001 monotherapy N = 23			
	Dose escalation N = 44	First expansion N = 69		Second expansion N = 31				
			N = 8*	N = 61		N = 13	N = 1	N = 4
CMP-001 Treatment:	CMP-001^	CMP-001 initially*	Diluted CMP- 001*	CMP-001 (10 mg)	CMP-001 (10 mg)	CMP-001 initially (10 mg)§	Diluted CMP-001 (5 mg)	Diluted CMP- 001 initially (5 mg)¶
ORR (95% CI):	22.7% (11.5%- 37.8%)	37.5% (8.5% - 75.5%)	11.5% (4.8% - 22.3%)	25.8% (11.9% - 44.6%)	15.4%	0%	0%	60%

*The first 8 patients in the combination therapy arm of the original ("First") expansion cohort were initiated on CMP-001 therapy using the standard drug product buffer at 10 mg. During their treatment course, on-site dilution of CMP-001 from the stock concentration of 6 mg/mL to a final concentration of 1 mg/mL (5 mg dose) was initiated using normal saline, and 6 of these 8 patients were switched to that formulation at various times during their treatment. The remaining 61 patients in the first expansion cohort were initiated on therapy using the on-site dilution of CMP-001 at 5 mg.

[^] dose levels: 1mg (N=3), 3mg (N=16), 5mg (N=9), 7.5mg (N=6), 10mg (N=10)

[§]1 patient in the monotherapy arm was initiated on treatment with CMP-001, but then switched to on-site dilution in saline at week 10

[¶]5 patients in the monotherapy arm were initiated on treatment with saline-diluted CMP-001, but then switched to undiluted CMP-001 at time points ranging from weeks 4 to 13

The purpose of this on-site dilution step was to increase the volume of injection, improving the distribution of the drug in the tumor. Initial studies showed no adverse effects of on-site dilution on CMP-001 efficacy. However, subsequent in vivo preclinical studies revealed that the on-site dilution step significantly reduces the anti-tumor efficacy of CMP-001, and so a second expansion cohort was enrolled, using the original undiluted buffer composition of CMP-001

to activate tumor-associated plasmacytoid dendritic cells, inducing an interferon-rich tumor microenvironment and anti-tumor CD8+ T cell responses.

Methods CMP-001-001 is an ongoing Phase 1b trial evaluating the safety and efficacy of CMP-001 in combination with pembrolizumab (Part 1; N = 144) or alone (Part 2; N = 23) in patients with advanced melanoma resistant to prior anti-PD-1 therapy (Tables 1). CMP-001 is administered IT into accessible lesion(s) either with, or without on-site saline dilution (table 1), and response assessed by RECIST v1.1. Monotherapy patients who progress can be rolled over onto combination therapy and continue on study. Baseline and on-therapy serum is analyzed for cytokines, and immunohistochemistry and RNA-Seq are performed on available tumor biopsies.

Results Adverse events (AEs) attributed to CMP-001 in combination with pembrolizumab or as monotherapy consisted predominately of transient low-Grade flu-like symptoms and injection site reactions: Grade 3+ related AEs were reported in 33% of patients treated with combination therapy and 22% of patients with monotherapy.

The Objective Response Rate (ORR) with undiluted CMP-001 in combination with pembrolizumab was 24% (18/75; 95% confidence interval: 15%-35% (table 1); on-site dilution of CMP-001 was associated with a substantial decrease in ORR to 12% (7/61; 95% confidence interval: 5%-22% (table 1). Three additional patients had a delayed partial response after an initial period of disease progression. Anti-tumor response was comparable between injected and uninjected lesions. The median duration of response to combination therapy has not been reached. The ORR to CMP-001 monotherapy was 22% (5/23; 95% confidence interval: 7%-44% (table 1); time from last anti-PD-1 therapy before CMP-001 was 1.5 to >20 months in responders; 3 of the patients responding to

CMP-001 monotherapy achieved PR at the first evaluation, but progressed by the second evaluation.

Serum and tumor biopsy translational studies in the patients receiving combination therapy supported the proposed mechanism of TLR9 activation and identified a possible association between induction of serum CXCL10 and response.

Conclusions IT CMP-001 alone and in combination with pembrolizumab appears well tolerated, can reverse resistance to anti-PD-1 therapy, and can produce deep and durable clinical responses in patients with advanced melanoma.

Ethics Approval CMP-001-001 was centrally approved by the WCG-WIRB, WIRB approval tracking number 20152597.

Poster Presentations

Biomarkers, immune monitoring, and novel technologies

P851

IDENTIFYING POTENTIAL PREDICTIVE BIOMARKERS FROM PLASMA EXOSOMES AND ADOPTIVE T CELLS THAT DIFFERENTIATE SHORT AND LONG-TERM METASTATIC NASOPHARYNGEAL CANCER SURVIVORS TREATED WITH CHEMOTHERAPY AND VIRUS-SPECIFIC T CELLS

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10.1136/LBA2019.5

Background The identification of biomarkers predicting a better outcome to adoptive T cell therapy is an emerging, still nascent field. We previously completed a phase 2 clinical trial of autologous adoptive EBV-specific cytotoxic T lymphocyte (CTL) immunotherapy following gemcitabine + carboplatin chemotherapy as first line treatment in 35 advanced incurable stage 4c nasopharyngeal carcinoma (NPC) patients. Here, we aim 1) to evaluate exosome proteins for potential specific predictive biomarkers of benefit to adoptive T cell therapy and 2) to investigate if a specific immunophenotype of our generated EBV targeting CTLs correlates with survival benefit.

Methods We isolated exosome from plasma samples by size exclusion chromatography and magnetic-based isolation technology, followed by fluorescence-activated cell sorting (FACS) analysis, Western blot analysis for immune-related checkpoint molecules, or mass spectrometry for exosomal peptide detection. All the generated CTLs were analysed by gene expression microarray as well as a FACS system with the use of T cell-specific 23-antibody panel for comprehensive immunophenotyping. The representative CTL samples were also subject to single-cell (sc) RNA-Seq with DNA barcoded antibodies for comprehensive integrated transcriptomics and immunophenotyping analysis.

Results Patients with overall survival longer than 2 years were grouped as long survivors and the remaining patients were grouped as short survivors. Differentially expressed immune-related checkpoint molecules, such as PD1, ICOS, and CD137, were detected in pre-treatment exosome of long and short survivors. Furthermore, more than 13,000 high confident and unique peptides which belong to 1,500 unique proteins were identified and quantified by mass spectrometry from the purified plasma exosome. Pathway enrichment analysis further showed that plasma exosome of the short survivors had significantly higher innate immune response-activating signal transduction-related peptides detected ($p\text{-value} = 4.81 \times 10^{-5}$). The transcriptomic analysis revealed that statistically lower expressions of *SELL* (CD62L) and *LEF1* were found in the EBV CTL of the short survivors, suggesting that the EBV CTL of short survivors were characterized by a lesser central memory T cell phenotype.

Conclusions Our data reports that specific pre-treatment plasma exosome proteins, and separately, transcriptomic profile of the generated baseline EBV CTLs prior to infusion into the patients correlate with patient survival, suggesting that they could be used together as biomarkers to predict outcome in the advanced NPC patients treated with this chemo-immunotherapy combination. More in-depth analysis of the immunophenotyping of all the CTLs and the representative CTLs' scRNA-Seq data will be presented at the meeting.

Acknowledgements This work was supported by Singapore government funding (National Medical Research Council (NMRC) – Open Fund - Large Collaborative Grant (OF-LCG) and National Cancer Centre Singapore Block Grant as well as in part by Tessa Therapeutics Ltd.

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TRANSCRIPTOMIC ANALYSIS OF DYSFUNCTIONAL CD8+ TILs IN MELANOMA IDENTIFIES BILE ACID AND MTOR PATHWAYS AS NOVEL POTENTIAL IMMUNOTHERAPY TARGETS

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10.1136/LBA2019.6

Background Coexpression of the immune checkpoint receptors PD-1 and TIM-3 is associated with dysfunction of tumor infiltrating lymphocytes in melanoma (MEL) and squamous cell carcinoma (SCC). To identify the mechanisms underlying this dysfunctional phenotype and to identify targets to rescue immune function, we performed transcriptional profiling of PD-1+TIM-3+CD8+ TILs sorted directly from MEL and SCC tumors in conjunction with immunologic phenotyping. We identified a number of novel dysregulated pathways and associated gene signatures in the PD-1+TIM-3+ subset that have not previously been reported in TILs, including bile acid metabolism, and demonstrated that these pathways are highly correlated not only to immune checkpoint receptor expression but also to MTOR pathway activation.

Methods Surgically excised melanoma tumors were dissociated and mononuclear cells were isolated by Ficoll gradient separation and cryopreserved. For sorting based on immune checkpoint receptor expression, a cocktail of monoclonal antibodies to the following targets, including viability dye was used: CD3, CD4, CD8, CD45RA, CD27, CCR7 PE, CD14, CD19, Live/Dead, BTLA, TIM-3, PD-1, CTLA-4, TIGIT and LAG-3. RNA was purified from 10,000 sorted cells using RNeasy Mini Kits (Qiagen), followed by RNASeq library generation using TruSeq Stranded Total RNA HT Kits (Illumina). Gene set variation analysis (GSVA) was used for pathway analysis, as well as linear regression modelling using limma (Bioconductor).

Results Transcriptomic analysis of CD8+ TILs revealed 4,228 genes ($P < 0.05$, t-test) as differentially expressed in TILs vs. functional peripheral CD8 T cells. Using a nonlinear dimensionality-reduction technique, we found that there were several unique clusters of cell surface marker expression that were present at significantly different frequencies in the TILs. We used pathway enrichment analysis and linear regression modelling to identify gene signatures that correlate with the progressive and coordinate expression of PD-1, TIM-3, and additional checkpoint receptors thereby driving progressive dysfunction on in TILs. The peroxisome and bile acid metabolism pathways were significantly enriched in the TIL transcriptome. Moreover, higher frequencies of events in dysfunctional clusters were strongly correlated with positive enrichment of the bile acid metabolism pathway and enhanced MTOR signaling.

Conclusions Transcriptomic analysis of PD-1+TIM-3+ CD8+ TILs identified novel candidate mechanisms of immune dysfunction in CD8+ TILs from patients with metastatic melanoma who fail immunotherapy. Our study identifies the bile