**Abstract 226**

Figure 1 t-SNE analysis of effector TIL populations identifies distinct, IFN-gamma and TNF-alpha-producing cells at early (day 11) and late (day 21) time points of subcutaneous MC38 growth. (a) Combined pseudocolored density plot of t-SNE parameters of viable, non-aggregated, CD45.2+, CD3+ and/or NK1.1+ cells from all time points and treatment conditions. (b) MFI values of clustering parameters from identified TIL populations were used in a hierarchical clustering analysis. Major clustering groups were then broadly identified as: TC, cytotoxic T cells; TH, helper T cells; gamma delta-like, gamma delta T cells or T cells clustering with gamma delta T cells; NK, natural killer cells; or O, other TIL. (c) Expression of effector molecules CD107a (top), IFN-gamma (middle), and TNF-alpha (bottom) among each identified TIL population. The extent of background signal for each effector molecule is denoted by a red-dashed FMO line. (d) A heat map of effector molecule MFIs overlaid onto the t-SNE analysis. (e) Analyses of TNF-alpha expression for P5 day 11. Included is the population location (upper left), TNF-alpha expression versus side-scatter (upper right), P5 frequency with check point blockade (lower left), and TNF-alpha MFI with check point blockade (lower right) (f) Analyses of IFN-gamma expression for P32 day 11. Included is the population location (upper left), IFN-gamma expression versus side-scatter (upper right), P32 frequency with check point blockade (lower left), and percent IFN-gamma with check point blockade (lower right).

TNF-alpha-producing NKT, which represent 53.5 ± 3.7% of all TIL. These are accompanied by modest frequencies of CD4+ and CD8+ TIL, producing low levels of IFN-gamma. After 21 days, NKT populations are reduced to 15.2 ± 1.5%, giving way to increased NK, CD4+, and CD8+ TIL, with increased IFN-gamma production. CB hastens this switch, markedly reducing NK to less than 20% of all TIL, downregulating TNF-alpha production across NKT and CD4+ T cell subpopulations, increasing CD4+ and CD8+ TIL frequencies, and significantly up-regulating IFN-gamma production at 11 days. CD107a expression patterns suggest degranulation is most associated with NK and NKT TIL (figure 1). NKT displayed no CD1d-restricted cytotoxicity against MC-38 ex vivo. However, CD1d overexpression on MC-38 significantly delayed tumor growth in vivo, suggesting early NKT activity may indirectly suppress tumor progression, but by what precise mechanism(s) is currently unknown.

**Conclusions** Despite evidence of an indirect benefit of early NKT activity, CB hastens a switch from TNF-alpha-driven NKT involvement toward a IFN-gamma-driven CD4+ and CD8+ T cell response in subcutaneous MC-38 tumors. These results corroborate earlier findings that CD4+ TIL are a major CB-responding population, and introduce a NKT/T cell switch that may be a key feature of the CB response in certain tumors.

**Ethics Approval** Animal experiments in this study were performed according to protocols approved by the University of South Florida’s institutional animal care and use committee (IACUC) committee, number R ISO0005710.

**REFERENCE**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0226

**227 USING 3D SPHEROID CULTURES TOWARDS PERSONALIZED EX VIVO PROFILING OF IMMUNE CHECKPOINT INHIBITOR EFFICACY IN MELANOMA AND NON-SMALL CELL LUNG CANCER**

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**Background** Immune checkpoint inhibitors (ICIs) have shifted the cancer treatment paradigm. Cancers such as melanoma and non-small cell lung cancer (NSCLC) demonstrate high tumor mutational burden and tumor neoantigen expression which renders them more responsive to checkpoint inhibitor blockade compared to other malignancies. Yet, 40–60% of metastatic melanoma patients do not have an initial response to ICI therapy and in NSCLC, PD-L1 expression, often a prerequisite for ICI treatment, does not definitively associate with ICI clinical response. Mechanisms of resistance often result from aberrant interactions between tumor and immune cells. Development of pre-clinical models that mimic the complex interplay between cells within the tumor microenvironment in a patient-specific manner are critical for accurate ex vivo profiling of ICIs. To improve immunotherapy predictive testing, we present a 3D spheroid culture system for testing personalized ICI efficacy.

**Methods** Cell lines co-cultured with T-cells from healthy donor peripheral blood mononuclear cells were used to optimize assay conditions and confirm ICI binding to target proteins. For ex vivo testing, primary melanoma or NSCLC tumor tissue from treatment naïve patients was dissociated and cultured as 3D spheroids using autologous immune cells. Development of pre-clinical models that mimic the complex interplay between cells within the tumor microenvironment in a patient-specific manner are critical for accurate ex vivo profiling of ICIs. To improve immunotherapy predictive testing, we present a 3D spheroid culture system for testing personalized ICI efficacy.

**Results** ICI binding to target proteins was measured across six ICIs, and no significant differences in concentration-dependent site occupancy within drug target classes was observed. However, differences in drug induced cytotoxicity across different tumor samples was detected even within the same drug target class. The immune composition of tumor samples that responded to ICIs displayed increased T-cell activation and increased IFNγ production. Furthermore, changes in PD-L1 and MHC-class I expression were detected which reflected ICI response. Finally, T-cell-dependent induction of tumor cell...
apoptosis was confirmed to be the observed mechanism of cytotoxicity within the 3D spheroid system.

Conclusions This work demonstrates that differences in ICI induced cytotoxicity can accurately be detected using our ex vivo 3D spheroid platform. The differences in therapy sensitivity can be related back to cell composition and function to potentially predict patient-specific drug response. Future correlation to patient clinical outcomes will be necessary for true clinical applications.

Acknowledgements N/A

Ethics Approval N/A

Tissue for this study was procured from commercial vendors who maintain strict ethical compliance, including fully de-identified materials and stringent IRB and Ethics Committee compliance.

Consent N/A

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0227

RADIOLOGICAL DYNAMICS AND RESISTANCE TYPES IN PATIENTS WITH ADVANCED MELANOMA TREATED WITH ANTI-PD-1 MONOTHERAPY

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Background The SITC Immunotherapy Resistance Taskforce recently defined primary and secondary resistance to anti-PD-1 therapy, yet there is little data that compares these two scenarios. In particular, detailed radiological dynamics of the different resistance types remain undescribed.

Methods We performed independent single-blind reevaluations of available radiological image data on a retrospectively assembled cohort of advanced melanoma patients (n=254, median follow-up 31.3 months, figure 1) treated with anti-PD-1 monotherapy initiated between Sept 2009 and Aug 2018 at both Massachusetts General Hospital and Peking University Cancer Hospital. Radiological characteristics and timing at multiple crucial radiological landmarks were analyzed and correlated with each other and with survival. As per the SITC Taskforce, primary resistance was defined as a best response of stable disease (SD) lasting less than six months or disease progression (PD), secondary as PD following an initial partial or complete response (PR/CR) or SD lasting 6 months or greater.

Results The most dramatic tumor reduction occurred within the first 3 months after anti-PD-1 initiation. A subpopulation of patients who had SD (28.6%, all with tumor shrinkage) experienced further tumor reduction and upgraded to CR/PR and 11.1% of patients with initial PR upgraded to CR. No patients without tumor shrinkage at the initial evaluation ultimately responded. Baseline tumor burden, response depth, timing of maximal response and PD pattern demonstrated great variation and were significantly correlated with each other and with survival. In multivariate analyses, deeper response depth was independently associated with a less widespread progression pattern, less involved organs, smaller target lesion size and slower tumor growth rate (all P<0.001) at PD, and longer post progression survival (PPS) (P=0.005). Compared to primary resistance, secondary resistance was correlated with less broad progression pattern, less tumor burden and slower tumor growth (all P<0.001). Patients with secondary resistance were more likely to receive further local/regional therapy (46.5% vs. 30.9%, P=0.07) rather than systemic therapy (27.9% vs. 56.9%, P<0.001), yet had a significantly longer PPS (HR 0.503, 95% CI, 0.288–0.879, P=0.02). Median PPS was not reached (95% CI, 11.8 months to not reached) for patients with secondary resistance and was 10.3 months (95% CI, 7.7–16.1) for patients with primary resistance (figure 2).

Conclusions Radiological dynamics were heterogeneous, yet significantly correlated with survival. Patterns of progression and PPS of the SITC Immunotherapy Resistance Taskforce defined primary and secondary resistance are different. This distinction may be important for the design of clinical trials targeting a PD-1 resistant population.

Abstract 228 Figure 1 MGH, Massachusetts General Hospital. PUCH, Peking University Cancer Hospital

Abstract 228 Figure 2 PPS of patients with primary resistance was significantly longer than those developed secondary resistance (P=0.009), with median PPS of 10.3 months (95% CI, 7.7 to 16.1) and not reached (95% CI, 11.8 to not reached), respectively

Ethics Approval This study has been conducted in compliance with local Institutional Review Board policies.