4200

Characterization of acquired resistant models to therapies targeting the PD-1/PD-L1 axis demonstrates model-dependent mechanisms

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Antineo company

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Introduction

Immune checkpoint inhibitors, such as antibodies directed against PD-1 and PD-L1, have been shown to produce durable responses in a subset of patients. However, many patients either are refractory or ultimately relapse due to acquired resistance mechanisms. As the underlying mechanisms of this secondary resistance are not well understood, we developed five syngeneic murine tumor models to characterize in vivo variants with acquired resistance to PD-1 and/or PD-L1 antibodies. Resistant in vivo models were obtained by serial treatment/reimplantation cycles in immunocompetent mice bearing MC38, MB49, MBT2, TyrNRas or RENCA tumors (Figure 1).

Results

We analyzed the tumor immune microenvironment in sensitive and aPD-1 and/or aPD-L1 resistant models by spectral flow cytometry (Figure 2). A panel of 29 markers was applied. Each resistant model displayed multiple modifications in the tumor immune infiltrate in comparison to the sensitive model, involving selected lymphoid and/or myeloid subpopulations.

Moreover, we performed an RNAseq analysis for all models. We observed alterations of the pathways already described as being modified in patients with disease progressing under ICI therapy, such as PTEN, INFy, PI3K / AKT or JAK1 / JAK2. However, this appears to be highly model-dependent, reflecting the heterogeneity observed in patients (Figure 3).

Generation of PD-1/PD-L1 resistant models

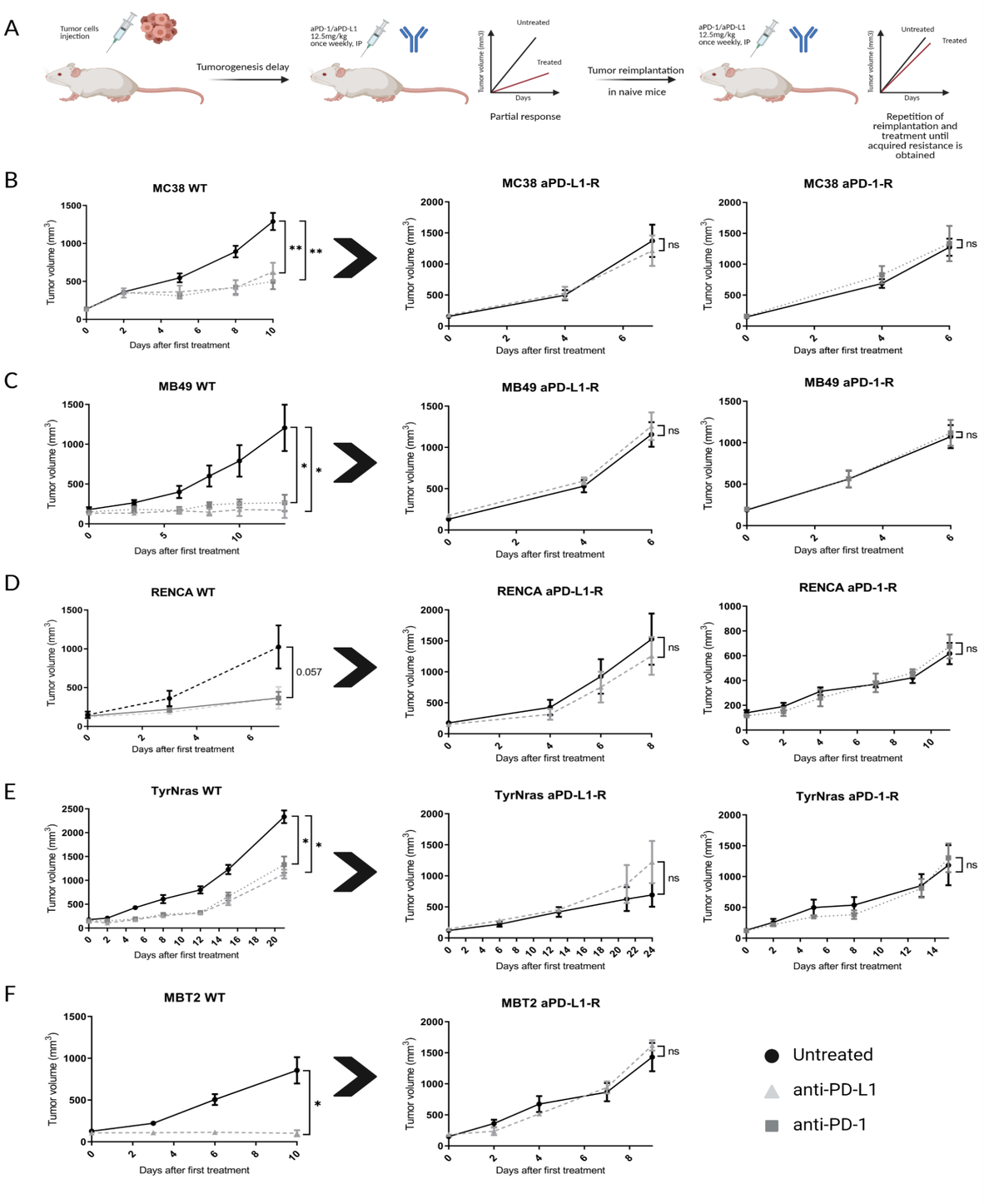

Figure 1. Wild type sensitive models were rendered resistant in vivo to aPD-1 and/or aPD-L1. For all models when tumors reached 150 mm3, mice were randomized and treated with aPD-1 (BioXCell, 12.5 mg/kg per week, IP) or aPD-L1 (BioXCell, 12.5 mg/kg per week, IP).

Immunophenotyping models comparison

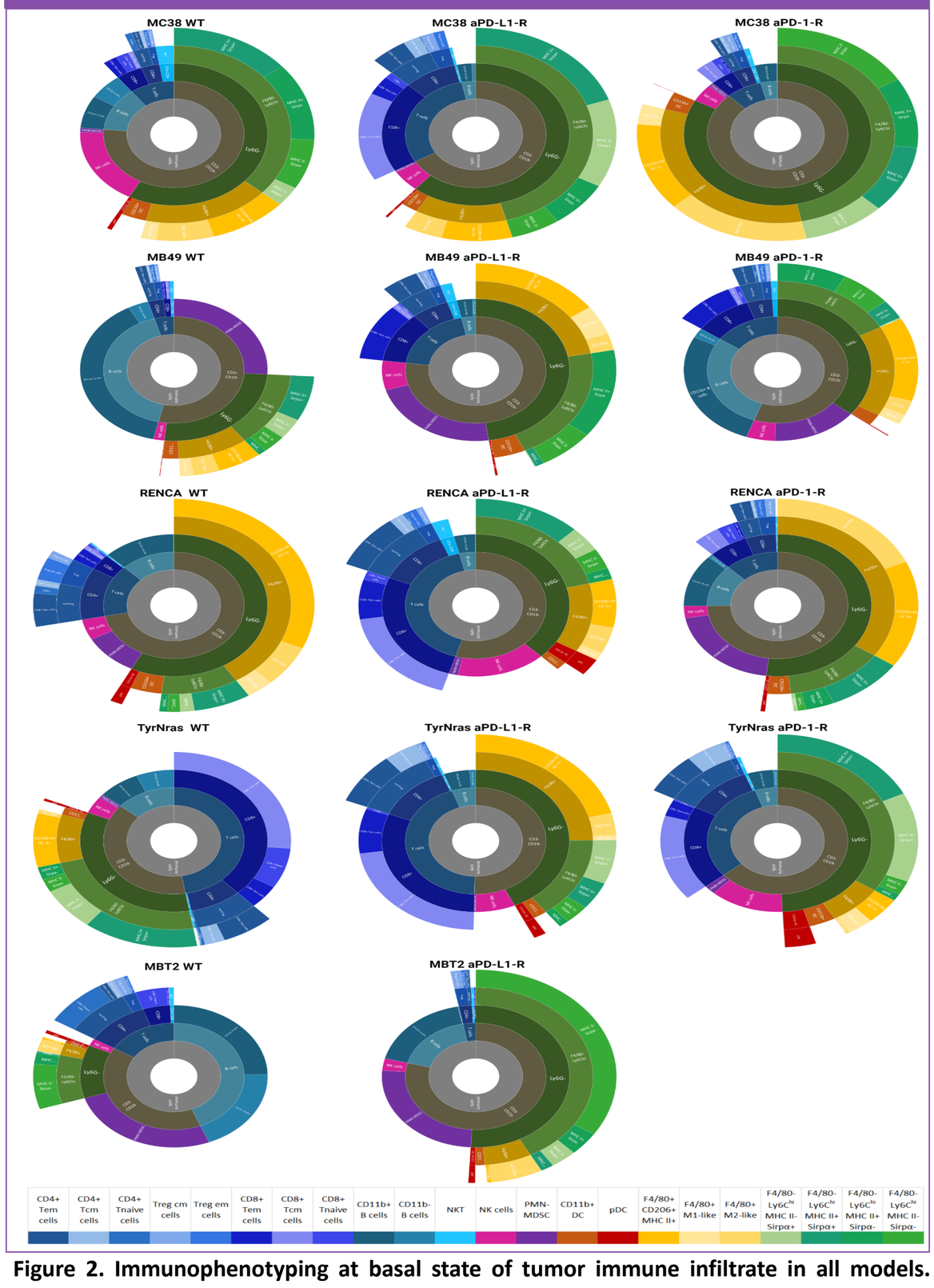

Figure 2. Immunophenotyping at basal state of tumor immune infiltrate in all models. Sunburst plots showing the proportion of CD45+ immune infiltration.

Deregulated pathways in resistant models

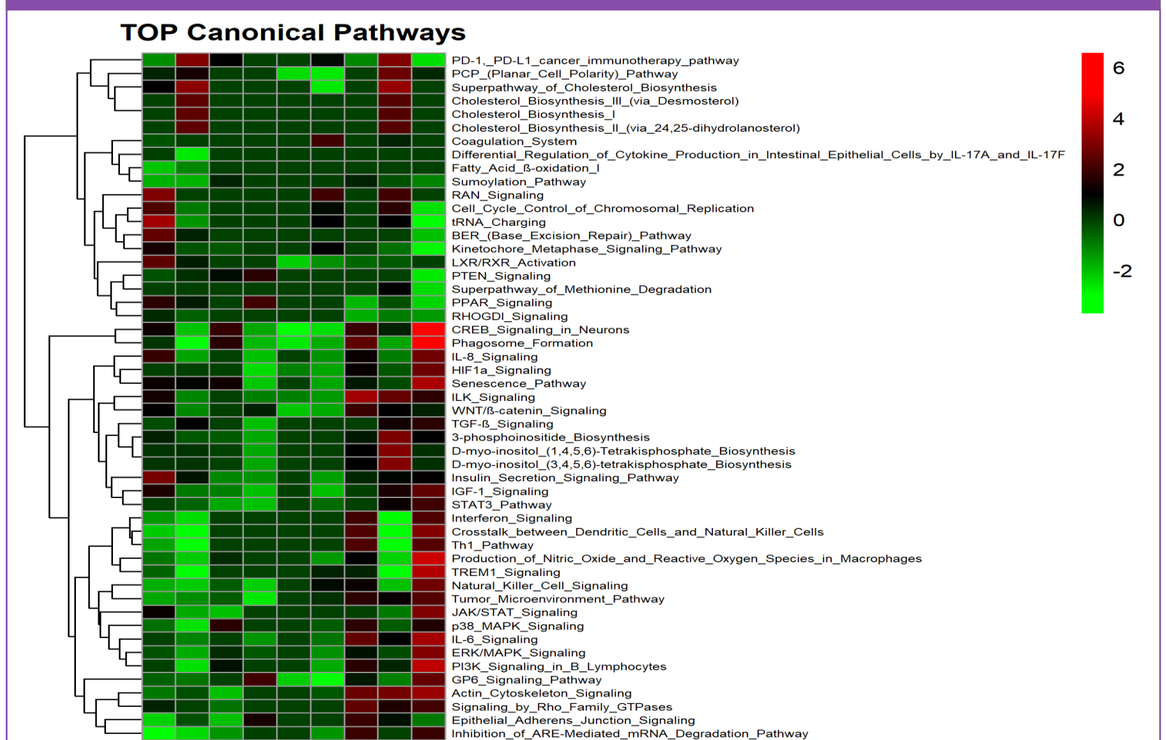

Figure 3. Heatmap displaying the prediction of deregulated pathways due to the acquisition of aPD-1 or aPD-L1 resistance. Top five up and down regulated pathways for each model. 5-fold z-score change and significant pvalue<0.05.

Results

In accordance with the up or down regulation of immune cells we tested therapeutic combination to overcome resistance. All combinations allowed to reverse resistance thought immune checkpoint inhibitor and induced a significant delay in tumor growth (Figure 4).

Efficacy of therapeutic combinations

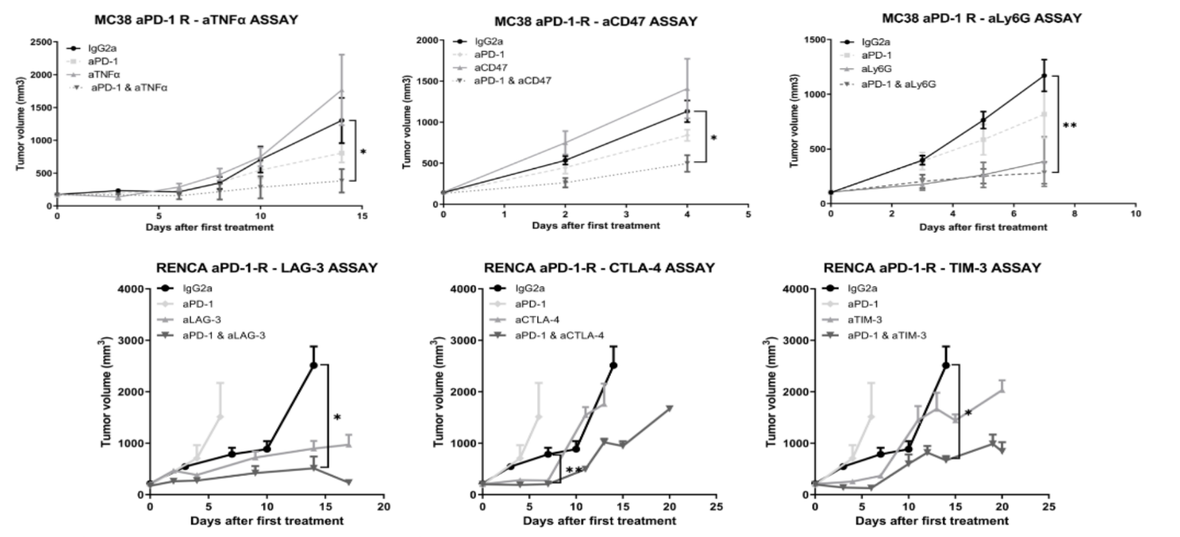

Figure 4. Efficacy of therapeutic combinations *in vivo* in preclinical aPD-1-R aPD-L1-R models. When tumors reached 150 mm3, mice were randomized and treated for each treatment in IP by anti-TNFa (BioXCell, 10 mg/kg per week), anti-CD47 (BioXCell, 20 mg/kg per week), anti-Ly6G (BioXCell, 2.5mg/kg once a day), anti-LAG-3 (BioXCell, 10 mg/kg twice a week), anti-CTLA-4 (BioXCell, 5 mg/kg twice a week), anti-TIM-3 (BioXCell, 12.5 mg/kg twice a week).

Conclusions

Acquired in vivo resistant models displayed strong diversity, both in terms of alterations of the tumor immune microenvironment and tumor gene expression profile. These variants may be used to probe the heterogeneity of resistance mechanisms observed in the clinic and contribute to the preclinical evaluation of combination regimens.

Perspective

Our model library, which may be enriched in the future with several other variants developed using the same methodology, provides an innovative tool to better apprehend the complexity and diversity of resistance to ICI and test resistance reversal strategies.

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