Phenotypic Human Primary Cell-Based Tumor Microenvironment Models for Evaluation of Drug Combinations for Immune Oncology

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Abstract
We have developed complex in vitro co-cultures of human primary cells to model disease states in a standardized format to enable phenotypic drug screening.

1. The Diversity PLUS™ panel of 12 BioMAP® systems has been validated to test agents and combinations with respect to predicting efficacy and safety.
2. Additional panels relevant to oncology consist of co-culture of human fibroblasts or endothelial cells, a cancer cell line, and immune cells.
3. These host tumor microenvironment (TME) models mirror tumor-associated immune suppression biology relevant for immune-oncology drug testing.
4. Drug effects on protein biomarkers in these models are measured and the resulting phenotypic signatures reveal how drugs will impact disease biology.

We evaluated multiple oncology drugs, including pembrolizumab (± PD-1) and paclitaxel (anti-mitotic) alone and in combination, across the broad non-cancer Diversity PLUS panel and in the oncology systems.

1. Paclitaxel is highly active in Diversity PLUS and TME systems with decreased levels of tissue and tumor cell-specific markers. Paclitaxel increased levels of cytokines produced by immune cells but only in the TME models.
2. While pembrolizumab was inactive in non-TME systems, it showed similar activity in the TME systems with increased levels of granulocyte colony stimulating factor (G-CSF), G-CSF, IL-10, IL-17A, and TNFα and decreased tumor cell markers.

In conclusion, phenotypic evaluation of drug combinations in complex human primary cell-based TME systems identifies therapeutic strategies warranting further clinical evaluation.

BioMAP Systems Model Human Disease Biology

The systems biology approach of the BioMAP technology is designed to capture the complexity of various pathologically relevant tissue microenvironments and provide information about how drugs behave in these settings, delivering actionable information on both compound efficacy and possible ADRs.

Results

Phenotypic drug screening.

1. pembrolizumab plus paclitaxel combination that are significantly different from both agents alone are summarized in the Differential Activities Matrix. Paclitaxel (30 μM) plus pembrolizumab at 100 – 1 ng/ml, had the most differential activities out of the 16 combinations tested.
2. Compared to the monotherapies, the combination of pembrolizumab (10 ng/ml) plus paclitaxel (30 μM) had increased IL-10 and decreased tumor cell markers CEACA6 and Keratin 20.
3. The heatmap shows the activities of all monotherapies and combinations tested. An * denotes the activities that are significantly different from the single agents.
4. Specific combinations showed potentiated anti-tumor activities including increased IL-6, IL-17A, and TNFα and decreased tumor cell markers CEACAM6 and Keratin 20.

Evaluations of Oncology Drugs Outside the TME

1. Pembrolizumab and paclitaxel were added to the Diversity PLUS panel of systems to determine their activity outside of the TME.
2. Pembrolizumab did not impact biomarkers in human primary cell-based systems without cancer cells, consistent with MIA of restoring immune responses in TME.
3. Paclitaxel is anti-proliferative (gray arrow) to endothelial cells and fibroblasts, among other cell types. In contrast to the TME models, paclitaxel shows reduced immune activation.

Conclusions

1. BioMAP systems model human disease biology to enable compound testing on human primary cells to predict clinical outcomes with respect to efficacy and safety.
2. Activities induced by compound specific to the TME include:
   - pembrolizumab restores immune activity with increased Granulocyte B, B& and TNFα.
   - Paclitaxel shows anti-tumor activity (CEACAM6 and Keratin 20) in addition to potentiated immune activity similar to pembrolizumab.
3. Outside of the TME:
   - Pembrolizumab was inactive suggesting that anti-PD-1 antibodies are not inherently inflammatory.
   - Paclitaxel was anti-proliferative and anti-inflammatory.
4. BioMAP systems enable screening of monotherapies and combinations in a robust and repeatable format in pathologically-relevant environments.
5. Testing the drug interactions of pembrolizumab and paclitaxel within the TME identified potentated anti-tumor activities by the combination relative to the monotherapies including decreased tumor cell markers and increased IL-6, IL-17A, and TNFα.
6. Successful combinations can increase efficacy with enhanced biological activity and may allow agents to be used at a lower concentration, lessening adverse events.

Summary

1. BioMAP Oncology systems mirror the complex host-tumor stromal and vascular microenvironments.
2. BioMAP Oncology systems provide a robust, reproducible and high-throughput approach to support the development of new agents, alone or in combination, for oncology and immuno-oncology.