Biochemical and Cell-based Methods of Investigating Early-Stage Candidates for Cancer Co-immunotherapies

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Abstract

Some cancers survive by hijacking cellular receptors that regulate immunity thereby contributing to an immunosuppressive tumor microenvironment (TME). Widely used immunotherapies, such as checkpoint inhibitors, target these same receptors and, while they do not completely eradicate tumors, they are often lifesaving, are limited as monotherapies by acquired resistance and toxicity. Current research on checkpoint inhibitors is aimed at finding combination therapies to overcome these limitations. These include a wide range of approaches such as expanded targeting of immunomodulatory receptors, small molecule inhibitors of therapeutically relevant enzymes, and selective killing of cancer cells through CAR T approaches. Here we describe biochemical and cell-based methods suitable for investigating early stage candidates in these areas of co-immunotherapy research.

LAG 3 As a Potential Target for PD-1 Cotherapies

Validation of LAG3:MHC II Reporter Assays

LAG3 is an immunosuppressive receptor associated with PD-1 to release T cell exhaustion, and neutralizing anti-Lag3 antibodies are effective in cancer models (2). MHC class II was the first ligand reported for LAG3, and the interaction is a target for potential immunotherapies (2). However, the ratio of MHC II in LAG3 control of immunity is highly debated (2-4).

The discovery of FGL1 as an MHCII independent ligand of LAG-3 is a recent breakthrough (5). FGL1 is overexpressed in many cancers making it a potential target for immunotherapy (5).

Here we demonstrate that Anti-Lag3 inhibits the LAG3:MHC interaction.

Janus Kinase/STAT Pathways

Validation of STAT Reporter Assays

Jak/STAT transduction factor pathways regulate cytokine receptors and downstream activation of both Jak and STAT activities are associated with several cancers (6). PD-1 is regulated by Jak/STAT pathways and Jak inhibitors reduce PD-1 expression in NSCLC tumor cells (7).

Regulation of PD-1 expression by Jak/STAT pathways make them targets for co-therapies with immune checkpoint inhibitors (7).

PARP and Kinase Combinations

Poly (ADP-ribose) polymerase (PARP) inhibitors have emerged as promising treatments for ovarian and breast cancer, most notably cancers for tumors with BRCA1/2 mutations (8).

However, toxicities associated with PARP inhibition are linked to PD-1 expression and increased DNA damages (8).

VEGFR

VEGFs are tyrosine kinases that play central roles in tumor angiogenesis (9).

Angiogenesis pathways are associated with DNA repair mechanisms and combinations of PARP and VEGFR inhibitors show improved clinical profiles (9).

PARP and VEGFR Biochemical Assays

Methods for Optimizing BCMA Targeting by CAR T

Validation of CAR T Reporter Assays

Early stage development of CAR T therapy requires optimization of target binding and effector function. BCMA is a popular target for CAR T therapy following success in clinical trials using first generation approaches.

Fully realizing the potential of immune checkpoint inhibitors in cancer immunotherapy requires overcoming both limited efficacy and unintended toxicity. Development of effective co-therapies is a promising approach. As proof of principle, we have demonstrated systems for developing potential drug candidates against known targets for co-therapies. These systems represent samples from larger panels including assays for multiple immunomodulating receptors, kinases, PARPs and epigenetic targets. Collectively these panels provide the means for enhancing both efficacy and selectivity of new early stage candidates.

Methods, References, & Further Reading

For experimental methods search for specific products at bpsbioscience.com bpsbioscience.com/immunotherapy bpsbioscience.com/kinase bpsbioscience.com/parp bpsbioscience.com/arr

References