Docket #: S19-355

CD9 and NK receptor ligands: a novel target and companion diagnostic for stratifying women with ovarian cancer most likely to benefit from NK immunotherapy

Ovarian Cancer Overview

Ovarian cancer is the most lethal gynecologic malignancy having a 5-year relative survival of 49% (2010-2016) in the US. Among women with cancer, ovarian cancer is the fifth leading cause of death. Globally, the incidence of ovarian cancer was estimated at 295,400 and 184,800 deaths (Globocan 2018). In the US, 21,750 new cases and 13,940 deaths are estimated to occur in 2020. The US prevalence in 2017 was estimated at over 233,400. Efforts to develop effective prevention and treatment of this disease are very challenging because 80% of patients are diagnosed with advanced disease (regional and distant metastases) which correlates to increased mortality. There is still an enormous need to develop effective and safe treatments that can surmount these obstacles. Addressing this need is of the highest priority for women with ovarian cancer.

A variety of modalities are used alone or in combination to treat patients including, surgery, chemotherapy, targeted therapy, hormone and immunotherapy. Cancer immunotherapy is a relatively recent paradigm-shifting treatment modality in oncology that, drives an immune response against cancer cells. Most immunotherapy approaches targeting a patient's T cells have shown remarkable success for a variety of malignancies. However, ovarian cancer is largely unresponsive, despite tumors having high levels of immune checkpoint proteins such as PD-1, CTLA-4, LAG-3 and PD-LI

Natural Killer (NK cells). are a type of white blood cell (lymphocyte) and a component of the innate immune system. They have an important role in rejecting tumors through their potent killing function. Several approaches are in development capitalizing on the killing function of NK cells. Nevertheless, tumors skillfully develop mechanisms to suppress NK cell cytolytic function.

Novel findings for diagnosis, prognosis, and treatment of ovarian cancer
Dr. Fantl and her laboratory conducted studies resulting in novel findings that may provide insight into the reasons that ovarian cancer tends to be unresponsive to immunotherapies. The Fantl Lab applied Mass cytometry (aka Cytometry by Time-Of-Flight (CyTOF)) to study the diverse cell types within primary ovarian tumor samples. The data revealed an intra-tumoral specific NK cell type that correlated with overall tumor mass. Remarkably, this NK cell type had features of decidual NK cells (nonkilling NK cells) that serve as an immune protectant, critical in the first trimester of pregnancy. Clearly, there could be no stronger form of immune suppression needed than for a mother-to-be to tolerate her fetus.

To determine how tumor cells could endow intra-tumoral NK cells with immune-suppressive properties, Dr Fantl's Lab performed a CyTOF analysis to measure NK receptor ligand expression levels present on tumor cells. These ligands represent a complex network of molecules that upon binding to NK cells promote their ability to kill tumor cells while binding of other ligands block tumor cell destruction. The NK receptor ligands were expressed in different combinations within three distinct ovarian intra-tumoral cell types each representing different states of tumor progression all within the same tumor sample. These three cell types represent: **i)** tumor cells within a primary tumor mass with cells all adhered to one another **ii)** cells transitioning to a metastatic state and **iii)** cells that have migratory and metastatic properties. The most suppressive combination of ligands was found in the metastatic cell types. These data provide insight into how tumor cells differentially regulate NK cell function to bypass killing activity and permit expansion of tumor cells.

One of the hallmark features of decidual NK cells is their expression of the surface protein CD9. The Fantl Lab demonstrated that in primary ovarian tumors, CD9 was abundantly expressed by both the intra-tumoral NK cells and ovarian tumor cells. Using reliable in vitro models of ovarian tumors, their Lab co-incubated ovarian tumor cells, representing the three stages of tumor progression with the NK-92 cell line chosen because it is used in the clinic in a variety of engineered forms. NK-92

cells do not express CD9. However, upon co-incubation with ovarian tumor cell lines, they acquired CD9 by a process called trogocytosis (greek: trogo – to gnaw) whereby a recipient cell extracts a membrane fragment from a neighboring cell, which in this case was the acquisition by NK-92 cells of an ovarian tumor cell membrane fragment incorporating CD9. Dr Fantl's Lab demonstrated that CD9 conferred NK-92 cells with immune-suppressive properties and furthermore with a CD9 blocking antibody, these properties were reversed such that NK-92 cells killed the ovarian tumor cells.

The presence of decidual-like NK cells has also been demonstrated in colorectal, melanoma, glioblastoma, lung and breast cancers (Albini and Noonan, Cancer Discovery 2020). Thus, the findings described for ovarian cancer can potentially have much broader applications.

State of Development
Proof of concept

Applications

- CD9 identified as a target to optimize NK cell immunotherapy. For example a patient could be pre-treated with a CD9 antibody before receiving NK immunotherapy. This blocking CD9 antibody could: i) prevent CD9 acquisition by the administered NK cells ii) activate intra-tumoral decidual-like NK cells to have a killing function.
- CD9 identified as a target to activate endogenous intratumoral decidual-like NK cells.
- NK receptor ligand expression as a companion diagnostic for multiple NK immunotherapies.
- Assisting more accurate prognosis based on Identification of novel biomarkers for stratifying ovarian cancer patients.
- Optimization of NK cell therapies on the basis of NK receptor ligand expression levels.
- Frequency of decidual-like NK cells as a biomarker for response to T cell immunotherapies.

Advantages

- CD9 offers a novel target for NK immunotherapy.
- NK receptor ligand expression levels on tumor cells offer a potential companion diagnostic for MK immunotherapy.
- Monoclonal CD9 antibody may be developed for commercialization.
- Technology can be extended to other cancer types (colorectal, melanoma, glioblastoma, lung, and breast cancers).
- High throughput quantitative screening of tumor samples for key prognostic markers determined by the investigations above.
- Prediction of specific therapies that could provide the greatest clinical efficacy on a per-patient basis.

Publications

- Gonzalez, V.D., et al. High-Grade Serous Ovarian Tumor Cells Modulate NK Cell Function to Create an Immune-Tolerant Microenvironment. *bioRxiv* 2020

Patents

- Published Application: WO2021050200
- Published Application: 20220326244

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