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Suppressing the suppressors

By Tim Fulmer, Senior Writer

Last week's termination of **Cell Genesys Inc.**'s Phase III trial of GVAX for prostate cancer is the latest in a long line of failures for cancer vaccine developers. A variety of molecular and cellular mechanisms have contributed to the disappointing outcomes, but a key driver remains the ability of the tumor microenvironment to suppress the host antitumor response.

New research now has identified a signaling pathway that is activated in immunosuppressive myeloid cells within the microenvironment, raising the possibility that this pathway could be targeted to overcome immunosuppression and boost cancer vaccine efficacy.

Two papers published in *The Journal of Experimental Medicine*[1] and *The Journal of Immunology*[2] build on previous work describing the role of myeloid-derived suppressor (MDS) cells in tumor progression.[3,4] MDS cells derive from hematopoietic precursor cells. They are recruited to tumors from the blood and bone marrow in response to tumor-secreted factors like IL-6.

MDS cells can induce host T cell tolerance,[5,6] which in turn can prevent cancer immunotherapy from eliciting a robust antitumor response.

"Together with regulatory T cells, MDS cells are likely one of the most important mechanisms used by tumors to suppress immune activity in the tumor microenvironment," said Paulo Maciag, senior scientist at cancer immunotherapy developer **Advaxis Inc.**

Until now, the signaling pathways underlying the production and accumulation of MDS cells within the tumor microenvironment were unclear. The new papers describe the role played by two members of the S100 family of calcium-binding proteins—S100A8 (MRP8; calgranulin A) and S100A9 (MRP14; calgranulin B)—in the maintenance of immunosuppressive MDS cells at the tumor site (*see* **Figure 1, "Blocking immunosuppression in the tumor microenvironment"**).

In the *JEM* paper, researchers at the **H. Lee Moffitt Cancer Center** and colleagues reported that production of MDS cells in tumor-bearing mice requires upregulation of S100A9.

In mice injected with lymphoma cells, knockout of S100A9 resulted in greater tumor infiltration of CD8 and CD4 T cells and less accumulation of MDS cells than that seen in wild-type mice. The knockout mice had higher rates of tumor rejection and lower tumor size than their wild-type littermates.

In the *Journal of Immunology* paper, a team at the **University of Maryland, Baltimore County** and the **Burnham Institute for Medical Research** reported that MDS cells synthesize and secrete S100A8 and S100A9 heterodimers that bind glycoprotein receptors on the surface of other MDS cells. This promotes accumulation of the immunosuppressive cells in the blood and lymphoid organs of tumor-bearing mice.

In mice with metastatic disease, an antibody targeting glycoprotein receptors lowered serum levels of MDS cells, S100A8 and S100A9 compared with those seen in mice that received a control antibody.

The authors of the *Journal of Immunology* article concluded that targeting the S100A8 and S100A9 heterodimer "may improve immunotherapy with cancer vaccines and other immune strategies that require an immune-competent host."

"Any cancer vaccine that relies on activation of CD4 and CD8 T cells will presumably benefit from prior neutralization of immunosuppressive MDS cells, and targeting the S100 proteins could help achieve this," noted Suzanne Ostrand-Rosenberg, corresponding author on the paper and chair of biochemistry at the University of Maryland, Baltimore County.

Combo platter

Although S100A8 and S100A9 might be good targets for preventing MDS cell accumulation at the tumor site, other factors also contribute to MDS cell recruitment. Thus, hitting the two proteins would likely only be one component of combination therapy.

"It's likely that targeting the S100A8/A9 heterodimer by itself would not be sufficient to abolish immunosuppressive MDS cells," said Ostrand-Rosenberg. "This is because other factors in the tumor microenvironment, including IL-6, IL-1β and prostaglandin E_2, can also stimulate MDS cell activity. Thus, some combination of factors, perhaps including the S100 proteins, will probably be targeted to block induction of immunosuppressive MDS cells."

Other researchers agreed on the need for combination immunotherapy.

"Based on evidence thus far, it's doubtful that inhibiting S100 proteins would be sufficient or even warranted in all cancers and tumor types," said John Vasilakos, VP of immunology at **Biothera**. "Nevertheless, we can envision a general strategy for boosting host immune response in the presence of immunosuppressive MDS cells. This might involve a therapy that combines an S100 protein inhibitor with an immunostimulatory molecule like a TLR agonist. The former would help reduce immunosuppression in the tumor microenvironment, while the latter would activate macrophages and dendritic cells to help drive an antitumor response."

Biothera's Imprime PGG, a soluble β-glucan derived from the cell walls of *Saccharomyces cerevisiae*, is in Phase I/II testing to treat metastatic colorectal cancer in combination with Erbitux cetuximab, an anti–epidermal growth factor receptor antibody marketed by **ImClone Systems Inc.** and **Bristol-Myers Squibb Co.** in North America and by **Merck KGaA** elsewhere. Imprime induces neutrophils to bind complement on the surface of antibody-targeted tumors, triggering a cancer cell–killing mechanism.

"It's important to better understand the full range of host immune

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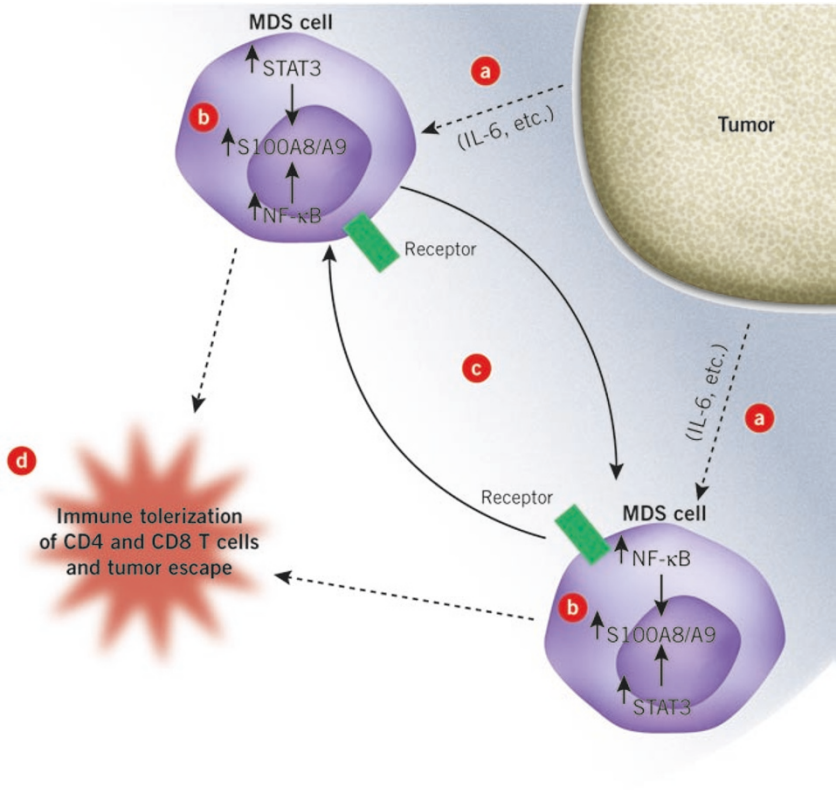

Figure 1. Blocking immunosuppression in the tumor microenvironment. Separate papers in the *Journal of Experimental Medicine* and the *Journal of Immunology* describe an immunosuppressive pathway driven by myeloid-derived suppressor (MDS) cells that could potentially be targeted to boost the efficacy of cancer immunotherapy.

[**a**] Tumors secrete multiple factors such as IL-6 that can prevent precursor immune cells from differentiating into mature, functional dendritic cells, granulocytes and macrophages. These immature myeloid cells are typically immunosuppressive and therefore referred to as MDS cells.

[**b**] The two papers highlight the central role played by the calcium-binding proteins S100A8 and S100A9 in the induction and maintenance of MDS cells in tumor-bearing mice. In both instances, upregulation of the S100 proteins via pathways that involve NF-κB and signal transducer and activator of transcription 3 (STAT3) leads to increased production and/or accumulation of MDS cells.

[**c**] MDS cells also secrete S100A8 and S100A9 heterodimers that potentially bind to receptors on other MDS cells to recruit them to the tumor microenvironment, thus setting up a positive feedback loop that can rapidly increase the number of MDS cells around the tumor.

[**d**] When MDS cells come into contact with CD8 and CD4 T cells, they induce host tolerization to the tumor, allowing the tumor to escape host immune surveillance and potentially contributing to tumor growth and cancer progression.

Potential therapeutic targets in this process include the tumor-derived factors in step [**a**], the S100 proteins in steps [**b**] and [**c**], multiple intracellular signaling molecules, including STAT3 and NF-κB and the as yet unidentified receptor or receptors that bind the S100 proteins.

cells that are downregulated or suppressed by MDS cells," said Vasilakos. "Cancer immunotherapy is generally a numbers game—the greater the number of functioning immune effector cells in the tumor microenvironment, the stronger the potential antitumor response. Abolishing MDS cells at the tumor site might help contribute to the success of cancer immunotherapies in the clinic."

Advaxis' Maciag thinks an alternative strategy is to induce MDS cells to differentiate into nonimmunosuppressive cells. "The immunosuppressive tumor microenvironment generally displays phenotypic plasticity," he said. "This means that in response to a particular stimulus, MDS cells can potentially transform into a cell type that no longer suppresses host antitumor response."

The company is pursuing that approach with Lovaxin C, a live *Listeria monocytogenes*–expressing HPV Type 16 E7 vaccine. This summer, the FDA placed a clinical hold on a proposed Phase II trial of Lovaxin to treat cervical intraepithelial neoplasia (CIN) related to preclinical, manufacturing, microbiologic, immunologic and clinical issues. Maciag said the company hopes to begin the Phase II trial early next year.

"Cancer immunotherapy is generally a numbers game—the greater the number of functioning immune effector cells in the tumor microenvironment, the stronger the potential antitumor response."
—John Vasilakos, Biothera

Figuring out the receptor

Although the *JEM* and *Journal of Immunology* articles help establish the central role S100 proteins play in the generation of MDS cells, the identity of the MDS cell surface receptor that binds and helps mediate their effects remains unclear. The identification of that receptor could provide an additional target to prevent accumulation of MDS cells in the tumor microenvironment.

Guided by work in other labs that had shown S100A8 and S100A9 are endogenous ligands of toll-like receptor 4 (TLR4) on immune cells,[7] the *JEM* authors speculated that hyperactivation of TLR signaling triggered by S100A9 might be at least partly responsible for producing immunosuppressive MDS cells.

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Table 1. NF-κB pipeline. Selected compounds in development that target NF-κB activity.

Company	Compound	Lead indication	Status
AnGes MG Inc. (Tokyo:4563)/Alfresa Pharma Corp.[A]	Decoy oligo	Atopic dermatitis	Phase II (Japan)
Cleveland BioLabs Inc. (NASDAQ:CBLI)	Curaxin (CBLC102)	Advanced hormone-refractory prostate cancer (HRPC)	Phase II
Hollis-Eden Pharmaceuticals Inc. (NASDAQ:HEPH)	Triolex	Type 2 diabetes	Phase III
Othera Pharmaceuticals Inc.	OT-551	Geographic atrophy in patients with age-related macular degeneration (AMD)	Phase II
Reata Pharmaceuticals Inc.	RTA 402	Chronic kidney disease	Phase II
VGX Pharmaceuticals Inc.	VGX-1027	Inflammatory disease	Phase I
4SC AG (Xetra:VSC)	4SC-301 (formerly SC75741)	Influenza; HCV	Preclinical
Reata	RTA dh404	Multiple sclerosis (MS); neurodegenerative disorders	Preclinical

[A]Partnered with Meyer Pharmaceuticals LLC in EU and U.S.

However, Yoshiro Maru noted that strategies to block S100 binding to TLR4 could potentially also block TLR4-lipopolysaccharide (LPS) interactions, which are essential for host immune response to infection. Maru, professor of pharmacology at the **Tokyo Women's Medical University**, said an ideal small molecule would be one that "selectively inhibited binding between TLR4 and S100A8/A9 without interfering with LPS."

LPS is an endotoxin that is a major component of the outer membrane of Gram-negative bacteria.

Maru and colleagues have shown that expression of S100A8 and S100A9 in the lung promotes the homing of tumor cells to premetastatic sites.[8]

Although the group on the *JEM* paper is looking at TLR4, the *Journal of Immunology* authors speculated that the putative receptor is likely a glycoprotein such as the advanced glycosylation end product—specific receptor (AGER; RAGE).

Indeed, Saeid Ghavami, professor of physiology at the **University of Manitoba**, told *SciBX* that he is collaborating with Walter Chazin at **Vanderbilt University** "to design a vaccine against peptide sequences of S100A8/A9. The vaccination strategy aims to prevent interaction between S100A8/A9 and receptors like RAGE, thus preventing activation of RAGE signaling in lung inflammation and potentially also in cancer."

> **"The immunosuppressive tumor microenvironment generally displays phenotypic plasticity."**
> ***—Paulo Maciag, Advaxis Inc.***

"It's already been reported that RAGE is expressed in large quantities on dendritic cells, and our lab has also shown that RAGE is targeted by S100A8/A9 on several types of cancer cells," said Marek Los, former faculty member at the University of Manitoba and founder and director of **BioApplications Enterprises**, a financial consulting firm.[9]

Los said small molecules that selectively interfere with S100A8- and S100A9-RAGE interactions might be a viable approach to neutralizing MDS cells.

Looking downstream

Although the identity of the surface receptor of the S100 proteins remains unknown, the two papers did identify signaling molecules downstream of the receptor that potentially regulate or interact with the proteins to modulate the immunosuppressive activity of MDS cells.

Targeting those signaling molecules could have an anti-immunosuppressive effect and neutralize MDS cells without directly targeting the S100 proteins or their receptor.

For example, the *JEM* authors identified a key role for the transcription activator signal transducer and activator of transcription 3 (STAT3) in the upregulation of S100A9 in MDS cells, whereas the *Journal of Immunology* authors found that S100A8 and S100A9 activated NF-κB signaling in the same cells.

"The findings in the *JEM* paper support our own strategy of targeting STAT3 signaling in tumor stromal cells like MDS cells, rather than in the tumor itself, to overcome mechanisms of tumor immune evasion," said Marcin Kortylewski, assistant research scientist at the **City of Hope National Medical Center**.

Kortylewski and Hua Yu, also at City of Hope, have shown that STAT3 upregulates multiple immunosuppressive factors and that selectively blocking STAT3 signaling in hematopoietic cells like MDS cells inhibits tumor growth and metastasis in mice.[10,11]

"Given the commonality of STAT3 activation in the tumor microenvironment, we believe a STAT3-targeting strategy has general applicability to boosting host antitumor response. A potential application would be to combine small molecule inhibition or siRNA silencing of STAT3 in MDS cells with immunostimulatory molecules like TLR ligands," said Kortylewski.

Maciag agreed that blocking mechanisms downstream of the MDS cell surface receptor could be a valuable strategy.

"Targeting upstream molecules like S100 is certainly worth exploring. However, the antitumor effects may not be so dramatic if other signaling pathways act to compensate for that inhibition," he said. "On the other hand, targeting downstream elements like NF-κB, where multiple pathways in carcinogenesis potentially converge, might yield a greater antitumor response. Of course, given the ubiquity of NF-κB, this approach would not be without risks of off-target toxicity."

A number of compounds are already in development targeting NF-κB activity (*see* **Table 1, "NF-κB pipeline"**).

Next steps

Dmitry Gabrilovich, corresponding author on the *JEM* paper, told *SciBX* he plans to study serum and tumor tissue levels of S100A9 as predictors for negative response to cancer immunotherapy in cancer patients.

"We will also continue our studies in mice to further characterize the

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role of S100 proteins in the development of immunosuppressive MDS cells. In particular, we want to get a better idea of how tumor induction and progression depend on levels of MDS cell activation," he said.

Gabrilovich is professor of oncologic sciences and molecular medicine at the **University of South Florida** and chair in cancer research at the H. Lee Moffitt Cancer Center.

Future work for Ostrand-Rosenberg and colleagues includes identifying mechanisms of chemoattraction that potentially induce MDS cells to migrate to tumors and determining whether proliferation of MDS cells primarily occurs in a particular region of the body, such as the bone marrow or lymph nodes.

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COMPANIES AND INSTITUTIONS MENTIONED

Advaxis Inc. (OTCBB:ADXS), New Brunswick, N.J.
BioApplications Enterprises, Winnipeg, Manitoba, Canada
Biothera, Eagan, Minn.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Burnham Institute for Medical Research, La Jolla, Calif.
Cell Genesys Inc., South San Francisco, Calif.
City of Hope National Medical Center, Duarte, Calif.
H. Lee Moffitt Cancer Center, University of South Florida, Tampa, Fla.
ImClone Systems Inc. (NASDAQ:IMCL), New York, N.Y.
Merck KGaA (Xetra:MRK), Darmstadt, Germany
Tokyo Women's Medical University, Tokyo, Japan
University of Manitoba, Winnipeg, Manitoba, Canada
University of Maryland, Baltimore County, Baltimore, Md.
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