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CD200: A solid argument

By Matthew Mikulski, Staff Writer

The two companies developing anti-CD200 antibodies to treat blood cancers—**Alexion Pharmaceuticals Inc.** and **Trillium Therapeutics Inc.**—might have had a new indication land in their laps. In a paper published in *The Journal of Clinical Investigation*,[1] researchers from the **University of North Carolina at Chapel Hill** (UNC) reported biomarker and mechanistic evidence that targeting CD200 could restore a latent antitumor immune response in melanoma patients.

The paper not only suggests that the use of CD200 antibodies could be expanded from liquid cancers into the solid-tumor setting but also hints that CD200 immunotherapeutics could have better side-effect profiles than antibodies against CTLA-4 (CD152), which are the most advanced melanoma compounds in the clinic.

Both CD200 and CTLA-4 inhibit T cell activity (*see* **Figure 1, "Suppressing T cell activity via CD200 and CTLA-4"**).

The receptor for CTLA-4 is expressed on T cells, and CTLA-4 inhibits T cell proliferation in the presence of B7-1 (CD80) or B7-2 (CD86) on antigen-presenting cells (APCs). The receptor for CD200 is located on dendritic cells (DCs), a type of APC. When bound, CD200 affects the ability of DCs to activate T cells.

Because antibodies against CD200 and CTLA-4 are both expected to have a positive effect on the immune response against cancer, the therapeutics could be used either in tandem or sequentially.

Screening for proteins

According to Norman Sharpless, assistant professor in the Department of Genetics at UNC and head of the research team that published the paper, his group came upon the role of CD200 as a result

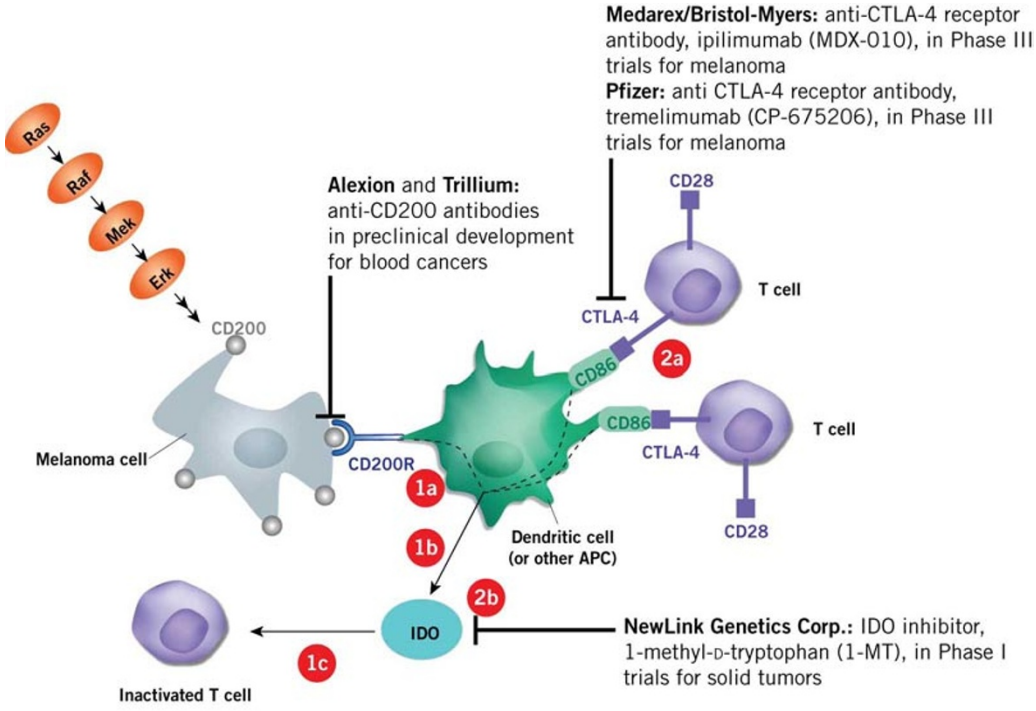

Figure 1. Suppressing T cell activity via CD200 and CTLA-4. CD200 and CTLA-4 (CD152) are both involved in suppressing T cell activity. Abnormal activation of the MAPK/ERK (Ras/Raf/Mek/Erk) pathway, notable in melanoma cells, leads to upregulation of hundreds of genes, including *CD200*. The binding of CD200 protein on cancer cells to its receptor on dendritic cells [**1a**] induces indoleamine-pyrrole 2,3-dioxygenase (INDO; IDO) expression [**1b**], resulting in the suppression of T cell activity and proliferation [**1c**]. CTLA-4 suppresses T cell activity indirectly by binding strongly to CD80 or CD86 on antigen-presenting cells (APCs), making CD80/CD86 inaccessible to CD28. Interactions between CD80/CD86 and CD28 would otherwise stimulate the activation of T cells [**2a**]. But like binding of CD200, CTLA-4 binding leads to IDO production, suppressing T cell activity [**2b**].

of their interest in the MAPK/ERK pathway. Increased MAPK/ERK activity has been associated with a variety of cancer types, and 80% of human melanomas harbor mutations that activate the kinase cascade, which is known to promote cell division, motility and survival through the upregulation of hundreds of genes.

In earlier studies, Sharpless and colleagues screened for proteins that were consistently upregulated in ERK-activated melanomas. CD200 was one of 82 targets that showed up. Because CD200 had already been shown to induce an immune inhibitory signal in humans, his group chose to focus their further studies on the protein.

The hypothesis that CD200 might be suppressing a clinically relevant immune response in melanoma patients was based on several lines of evidence, Sharpless said. Functional T cells that recognize melanoma antigens can be recovered from patients with metastatic disease, which suggests that the immune system is trying to fight the disease, but something is getting in the way.

Also, the development of vitiligo, a skin condition believed to result from autoimmunity, is associated with improved survival in patients with metastatic disease. Such patients might possess immune systems that are not entirely silenced by CD200 or CTLA-4 signaling.

"All these things lead you to believe that there might be a latent immune response in melanoma," Sharpless said.

The group first conducted *in vitro* studies confirming that the CD200 mRNA and protein are indeed regulated by the MAPK/ERK pathway. Next, they showed that 67% of resected melanocytic lesions, but no healthy cells, overexpressed CD200.

In addition, CD200 mRNA correlated with disease progression and also was detected in nevi (moles). If dysplastic and numerous, these lesions indicate a high risk for skin cancer. Knockdown of the human CD200 ligand using short hairpin RNA in high CD200–expressing melanoma cell lines impaired the ability of the cancer cells to inhibit T cell proliferation.

Finally, the *JCI* paper examined CD200 in other cancers and found that mRNA expression was more abundant in melanoma than in blood, breast, colon and lung cancers. "If you're going to try CD200-blocking antibodies, melanoma should be at the top of your list," said Sharpless.

Clinical transition

Sharpless's point has not been lost on Trillium Therapeutics, which has an anti-CD200 antibody in preclinical development. "We've thought about starting our Phase I in leukemia, but here they make good arguments for looking at melanoma," CEO Niclas Stiernholm told *SciBX*. Trillium, which has an undisclosed partner for the antibody, has not yet committed to a specific indication.

According to Stiernholm, research papers from as early as 2001 suggested that CD200 was involved in blood cancers[2]. But he said that melanoma has been under the radar because academic scientists have only recently developed an interest in the role of CD200 in solid tumors.

"Nobody has systematically looked at as many different melanomas as this paper," Stiernholm said. "This confirms that another very serious cancer could be treated by blocking CD200."

The other biotech with a preclinical anti-CD200 antibody, Alexion Pharmaceuticals, is pursuing chronic lymphocytic leukemia (CLL). Steve Squinto, EVP and head of research, said the *JCI* paper offers strong data correlating CD200 expression with a wide variety of melanoma lines as well as with human disease progression.

Squinto said Sharpless has contacted Alexion asking if he could collaborate to test the company's anti-CD200 antibody in melanoma. Squinto said Alexion is focused on finding the optimal dosing and the safety profile of its antibody in CLL and that the company has yet to engage in any formal discussions regarding other indications.

Testing other approaches

If Trillium, Alexion or another company picks up the ball in melanoma, they would join at least 16 players that are tackling the disease via immune modulation (*see* **Table 1, "Targeting melanoma"**).

Although relatively few compounds currently in the clinic are designed specifically to restore T cells or boost T cell activation, they do include some of the most advanced melanoma therapeutics—anti-CTLA-4 antibodies—in addition to the earlier-stage target indoleamine-pyrrole 2,3-dioxygenase (INDO; IDO).

Pfizer Inc., as well as **Medarex Inc.** and partner **Bristol-Myers Squibb Co.**, have anti-CTLA-4 antibodies in Phase Ill testing to treat metastatic melanoma. Pfizer is running a 650-patient Phase Ill trial of tremelimumab (CP-675,206; formerly ticilimumab) as a monotherapy.

In December, Medarex and Bristol-Myers reported that their ipilimumab missed the primary endpoint of a best objective response rate of ≥10% in a 150-patient Phase II monotherapy trial. Nevertheless, the biotech and the pharma companies said they hope to submit a BLA for approval of ipilimumab in second-line melanoma based on the results of that study plus two other Phase II trials.

MDX-1379 is a peptide-based melanoma vaccine developed specifically as an adjuvant to ipilimumab.

The companies also have two ongoing Phase III studies looking at ipilimumab in both first-line and salvage therapy. Both are in patients with unresectable tumors. The first-line study is in combination with dacarbazine. The other study has three treatment arms: ipilimumab monotherapy, MDX-1379 monotherapy and a combination of the two.

Although both anti-CD200 and anti-CTLA-4 therapies should help restore the activity of the innate immune response to cancer, Sharpless thinks that CD200 might have a safety advantage. He noted that CTLA-4–deficient mice exhibit a lethal lymphoproliferative disorder, whereas CD200 knockout mice have a much less severe autoimmune phenotype.

CD200 knockout mice are more susceptible to various models of experimental autoimmune diseases, such as encephalitis or collagen-induced arthritis, but are not overtly sick, according to Sharpless. "Loss of both molecules fosters autoimmunity, but we are hoping the CD200-based approach will be more of a 'smart bomb'," he said.

Patients receiving anti-CTLA-4 antibodies in clinical trials have had immune-related adverse events including rashes, colitis, diarrhea and pruritis.

Ultimately, Sharpless believes that immunomodulatory agents will

Table 1. Targeting melanoma. Alexion Pharmaceuticals Inc. (NASDAQ:ALXN) and Trillium Therapeutics Inc. have anti-CD200 antibodies in preclinical development for blood cancers, but a paper in The Journal of Clinical Investigation suggests that the target also plays a role in melanoma by blocking the immune response to the disease. Thus, CD200 could potentially join the list of melanoma targets that companies are pursuing to create immune-modulating melanoma therapeutics. Selected targets are listed below.

Target	Company	Product [molecular target]	Status
CTLA-4 (CD152)	Medarex Inc. (NASDAQ:MEDX)/ Bristol-Myers (NYSE:BMY)	Ipilimumab (MDX-010) [CTLA-4]	Phase III
	Pfizer Inc. (NYSE:PFE)	Tremelimumab (CP-675,206; formerly ticilimumab) [CTLA-4]	Phase III
Dipeptidyl peptidases (DPP)	Point Therapeutics (NASDAQ:POTP)	Talabostat (PT-100) [DPP-8; DPP-9; fibroblast activation protein]	Phase II[A]
Heat shock proteins (Hsp)	Synta Pharmaceuticals Corp. (NASDAQ:SNTA)/GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	Elesclomol (STA-4783) [Hsp70]	Phase III
	Kosan Biosciences Inc. (NASDAQ:KOSN)	Tanespimycin (KOS-953) [Hsp90]	Phase II
Indoleamine-pyrrole 2,3-dioxygenase (INDO; IDO)	NewLink Genetics Corp.	1-Methyl-D-tryptophan	Phase I for solid tumors[B]
Interleukin (IL) receptors	Novartis AG (NYSE:NVS; SWX:NOVN)	Proleukin aldesleukin [L-2 receptor]	On market
	ZymoGenetics Inc. (NASDAQ:ZGEN)/ Novo Nordisk A/S (NYSE:NVO)	Interleukin-21 [IL-21 receptor]	Phase II
	Introgen Therapeutics Inc. (NASDAQ:INGN)	INGN 241 [IL-24 receptor]	Phase II
	Cytheris S.A.	CYT107 [IL-7 receptor]	Phase I/II
	Vical Inc. (NASDAQ:VICL)	VCL-IM01 gene therapy [IL-2]	Phase I
Interferon (IFN) receptors	Schering-Plough Corp. (NYSE:SGP)	Intron-A interferon alfa-2b [IFN receptor]	On market
	Enzon Pharmaceuticals Inc. (NASDAQ:ENZN)/Schering-Plough	PEG-Intron peginterferon alfa-2b [IFN receptor]	Phase III
Major histocompatibility complex class II (MHC II)	Immutep S.A.	ImmuFact IMP321[MHCII] plus antigen	Phase I/II
Tumor necrosis factor-α (TNF-α)	GenVec Inc. (NASDAQ:GNVC)	TNFerade gene therapy [TNF-α]	Phase II
Toll-like receptors (TLR)	Pfizer (compound from acquisition of Coley)	PF-3512676 (CpG 7909) [TLR9] plus tremelimumab [CTLA-4]	Phase I

[A]Talabostat clinical program on hold after interim analyses of a pair of Phase III trials to treat non–small cell lung cancer showed a lack of efficacy. [B]Melanoma not specified as an indication being pursued.

need to be given in tandem with other agents to be effective. "I have been doing oncology long enough to know that it is unlikely that any new single agent, no matter how promising, will turn out to solve all our problems in melanoma," he said.

Targets downstream of CD200, such as IDO, could be prime candidates.

Andrew Mellor, director of the Immunotherapy Center at the **Medical College of Georgia** (MCG), told *SciBX* that the binding of CD200 triggers the release of IDO, which his group and others have studied in certain regulatory DCs[3].

Mellor, who has studied the effects of inhibiting IDO, also believes that blocking one inhibitory pathway is not likely to be sufficient in treating cancer. "We view IDO inhibitors as a vaccine adjuvant—they have an effect on their own, but would be much more effective when used in combination with other reagents," he said.

NewLink Genetics Corp. has rights to intellectual property from MCG covering IDO inhibitors to treat cancer. NewLink recently started a Phase I trial of lead candidate 1-methyl-D-tryptophan (1-MT) in patients with metastatic or refractory solid tumors.

In addition to combinations with downstream targets, anti-CD200 therapeutics might also be paired with treatments that indirectly stimulate an innate immune response, such as elesclomol (STA-4783) from **Synta Pharmaceuticals Inc.** Anti-CD200 agents might also be used in combination with treatments that stimulate both arms of the immune system, such as Allovectin-7 from **Vical Inc.**

Indeed, Robin Jackman, SVP of business operations at Vical, expects that anti-CD200 or anti-CTLA-4 antibodies would likely be synergistic with Allovectin-7. A lipid-complexed form of the gene encoding HLA-B7–mismatched transplantation antigen is in Phase III testing to treat metastatic melanoma. The product does not contain tumor-associated antigens. Rather, Allovectin-7 works by making the tumor appear like a transplant from an incompatible tissue donor.

Finally, traditional vaccines that contain tumor-associated antigens also could potentially benefit from the removal of T cell blockades. There are a number of such vaccines in the clinic *(see* **Table 2, "Melanoma vaccines"**).

Although melanoma will likely be the first solid tumor in which CD200 antagonism is explored, the target's utility in solid cancers could have additional applications. Shortly after the UNC group

Table 2. Melanoma vaccines. In addition to the biotech and pharma companies developing targeted immune modulators for melanoma, a host of companies are tackling the disease via therapeutic vaccines. Selected vaccines for melanoma that are in clinical development are listed below.

Company	Product	Product description	Status
Avax Technologies Inc. (OTCBB:AVXT)	M-Vax	Autologous haptenized tumor cell vaccine	Phase III
Antigenics Inc. (NASDAQ:AGEN)	Oncophage vitespan	Vaccine containing heat shock proteins isolated from the patient's cancer cells	Phase III[A]
Medarex Inc. (NASDAQ:MEDX)	MDX-010 plus MDX-1379	Anti-CTLA-4 antibody (MDX-010) plus gp100 peptide vaccine (MDX-1379)	Phase III
Vical Inc. (NASDAQ:VICL)/ AnGes MG Inc. (Tokyo:4563)	Allovectin-7[B]	Gene encoding HLA-B7–mismatched transplantation antigen complexed with lipid	Phase III
BioVex Inc.	OncoVEX GM-CSF	Modified herpes simplex virus (HSV-1) encoding GM-CSF	Phase II
Cytos Biotechnology AG (SWX:CYTN)	CYT004-MelQbG10	Vaccine consisting of a modified fragment of the Melan-A/MART-1 protein coupled to the carrier QbG10	Phase II
IDM Pharma Inc. (NASDAQ: IDMI)/sanofi-aventis Group (Euronext:SAN; NYSE:SNY)	Uvidem	Vaccine comprising autologous dendritic cells loaded with melanoma tumor cell lysates	Phase II
Norwood Immunology Ltd. (LSE:NIM)	Melanoma cancer vaccine	Vaccine containing antigens gp100 and melanoma antigenic epitope (MAGE-3) plus leuprolide	Phase II
Oxford BioMedica plc (LSE:OXB)	Hi-8 MEL	Plasmid DNA plus modified vaccinia virus Ankara (MVA) vector expressing seven cytotoxic T cell epitopes derived from five melanoma antigens	Phase II
MolMed S.p.A./Takara Bio Inc.	M3TK	Autologous T lymphocytes genetically engineered to express tumor antigen MAGE-3	Phase I/II
NewLink Genetics Corp.	HyperAcute-Melanoma	Vaccine containing killed melanoma cells that contain a mouse gene which causes the production of a foreign pattern of carbohydrates on the cell surface	Phase I/II
Mannkind Corp. (NASDAQ:MNKD)	MKC1106-PP	Plasmid DNA encoding preferentially expressed antigen of melanoma (PRAME) and prostate-specific membrane antigen (PSMA) plus synthetic peptide analogs of PRAME and PSMA	Phase I

[A]Antigenics said it is focusing its efforts on development of Oncophage for the renal cell carcinoma indication. [B]Uses an immune-activating antigen that is not derived from cancer cells.

published its results, support for testing CD200 in other solid tumors came from separate papers published by scientists at Alexion and the **University of Montpellier**. The groups showed that CD200 was upregulated in renal cell cancer, head and neck cancer, ovarian cancer, testicular cancer, malignant mesothelioma and colon cancer[4,5].

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COMPANIES AND INSTITUTIONS MENTIONED

Alexion Pharmaceuticals Inc. (NASDAQ:ALXN), Cheshire, Conn.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Medarex Inc. (NASDAQ:MEDX), Princeton, N.J.
Medical College of Georgia, Augusta, Ga.
NewLink Genetics Corp., Ames, Iowa
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Synta Pharmaceuticals Corp. (NASDAQ:SNTA), Lexington, Mass.
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