Immuno-oncology at the crossroads: where are we headed after PD-1 blockade?
Immuno-oncology agents are being developed with the objective of harnessing a cancer patient’s immune system to eliminate tumors principally by inducing T-cell attack. Few therapies such as high dose IL-2 for Melanoma and kidney cancer had previously provided proof of principle that the body’s T-cells could be enlisted to attack tumors. However, it was not until antibodies targeting the checkpoint proteins cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death-1 (PD-1) achieved notable clinical successes that the entire cancer therapeutic community became convinced that stimulating anti-tumor immunity was feasible. Despite these successes a large fraction of patients remains unresponsive to these therapies. Importantly, a number of tumor types are inherently nonresponsive to these agents. Thus, clinical immuno-oncologists are looking toward adding to their armamentarium newer immunotherapeutics, especially as combinations with established standard of care (SoC) regimens, or with other emerging and novel agents.

There are currently over 50 newer immuno-oncology mechanisms of action (MoAs) being targeted with drugs that are in various stages of clinical development. These MoAs are directed against several distinct steps of a well-recognized immunologic cascade that has been rendered dysfunctional by a growing tumor. Successful immune surveillance against incipient tumors is thought to occur when antigen-specific T cells acquire effector function, expand clonally, traffic to sites of tumor cell growth and specifically lyse them. However, frequent expression/overexpression of checkpoint or inhibitory receptor proteins, results in functionally ‘exhausted’ T-cells, thereby helping tumors evade the immune system.

Prominent among the newer classes of cancer immunotherapeutics are monoclonal antibodies that target these checkpoint/inhibitory proteins. These checkpoint proteins include CTLA-4, PD-1, LAG-3, T-cell immunoglobulin (Ig) domain and mucin domain 3 (TIM3), among others. Antibodies against CTLA-4 (ipilimumab) and PD-1 (pembrolizumab and nivolumab) have already secured regulatory approval as monotherapies. Recently the combination of ipilimumab and nivolumab was also approved by the US FDA in Melanoma. In addition, Phase III trials evaluating the combination have also been initiated in NSCLC, SCLC, RCC, and glioblastoma based on efficacy observed in early phase trials. Efforts are currently underway to examine antibodies that block the other checkpoint proteins as well.

**Targets on Cancer Immunity Cycle**

- DAMPs and/or PAMPs
- PRR
- DC
- Peptide–MHC class I
- PDL2
- PD-1
- TIM3
- GITR
- GITL
- 4-1BB
- 4-1BBL
- CD27
- CD40
- CD70
- IL-12
- Type I IFNs
- Galectin 9
- HMGB1
- PtdSer
- Dying tumor cell
- CD80/CD86
- CD70
- E-cadherin
- KLRG1
- CD80
- CD86
- CTLA4
- TCR
- CD4
- CD8
- TCR
- MHC class I
- TCR
- MHC class II
- Perforin
- Granzyme
- Ox40
- Ox40L
- IL-12
- TIM1
- LAG3
- CD4
- GITR
- GITL
- 4-1BB
- CD27
- CD40
- CD70
- IL-12
Another class of antibodies being developed targets stimulatory co-receptors that belong to the TNF receptor superfamily (TNFRSF) including CD137 (4-1BB/TNFRSF9), CD27 (TNFRSF7), CD40 (TNFRSF5), GITR (CD357/TNFRSF18) and OX-40 (CD134/TNFRSF4). Full activation of naive T-cells requires a ‘second’ signal through CD28 in addition to the cascade initiated by the engagement of the TCR with its cognate peptide antigen in the context of the major histocompatibility complex (MHC) (which is considered the first signal). Further activation of the T-cell is dependent upon the above-mentioned co-stimulatory ligands and their receptors. In contrast to agents that target checkpoint proteins, the objective of the antibodies against TNFRSF members is to activate these receptors and they are therefore considered agonists.

In addition to these two broad classes of antibodies there are several small molecules being developed that target chemokine receptors and toll-like receptors (TLRs) among others. These also have the potential to engage various targets in the cancer immunity cascade and thereby facilitate an effective T-cell mediated antitumor immune response. Other immune-oncology MoAs that have assets undergoing clinical development include broad classes such as vaccines, oncolytic viruses, CAR-Ts and other T-cell therapies.

While sponsors continue to hold many drug development and mechanistic details proprietary, a few overarching themes are beginning to emerge from some of the emerging MoAs.

1. Antibodies against newer checkpoint proteins are being tried more often as combinations; monotherapy trials are primarily in hematological malignancies. In this category, agents targeting the inhibitory receptors LAG-3 (lymphocyte activation gene 3) and KIR (killer cell Ig-like receptors) appear to be the most advanced in the clinic.

LAG3: LAG3 is 20% identical to the CD4 gene, binds MHC class II molecules, and is only expressed several days following T-cell activation. LAG3 is found to be upregulated on exhausted T cells and its role in the negative regulation of T-cell function is well-known. Preclinical tumor models utilizing anti-LAG3 show enhanced activation of antigen-specific T cells at the tumor site and reduced tumor growth. LAG3 is also expressed on activated B cells in a T-cell dependent manner although its function there remains to be established. Two antibodies targeting LAG-3 (BMS986016, BMS and LAG-525, Novartis) are currently under evaluation for their single agent activity in Multiple Myeloma (MM), indolent Non-Hodgkins Lymphoma (iNHL), Diffuse Large B-Cell Lymphoma (DLBCL) and Hodgkins Lymphoma (HL) while their combinations with anti-PD-1 agents are under evaluation in solid tumors (NSCLC, Ovarian Cancer, Melanoma, Head and Neck Cancer, Renal Cell Carcinoma (RCC), Esophageal Cancer and MSI High Colorectal Carcinoma (CRC)). Preclinical studies have also revealed extensive coexpression of PD-1 and LAG-3 on tumor-infiltrating CD4(+) and CD8(+) T cells in several tumors. Dual anti-LAG-3/anti-PD-1 antibody treatment have also been shown to cure established tumors in mice that were largely resistant to single antibody treatment.

- Anti-LAG3 and anti-PD-1 dual treatments demonstrate synergy in preclinical tumor models.
- Whereas dual LAG3/PD-1 knockout mice demonstrate increased survival from clearance of transplanted tumors they also reveal autoimmune infiltrates in multiple organs.
- Clinical data from anti-LAG3 single agent and combination strategies are awaited; appropriateness of concurrent versus sequential treatments will be key considerations in combining anti-LAG3 and anti-PD-1 agents.

KIR: KIRs include inhibitory and activating receptors expressed on mature NK cells whose ligands are HLA molecules. KIRs are also expressed on CD8+ T-cell subsets in some tissues. When KIRs are ligated to their cognate ligands on autologous cells NK cells are educated to distinguish ‘self’ from ‘missing self’. Educated NK cells thus trigger cytotoxicity when they encounter malignant cells that either don’t express these ligands or express polymorphic HLA variants. NK cells are rendered tolerant when inhibitory KIRs when ligated to self-HLA ligands (HLA-C) are rendered tolerant. Inhibitory KIR proteins include the long-tail family members KIR2DL1, KIR2DL2, KIR2DL3. To exploit this pathway pharmacologically, the fully human mAb anti-KIR 1-7F9 (IPH2101, Innate Pharma) was first generated, and then another version of this mAb was developed with a stabilized
hinge (lirilumab, BMS-986015 / IPH2102, BMS/Innate Pharma). Lirilumab and lirilumab mAbs cross-react with KIR2DL1, -L2, and -L3 receptors and impair their inhibitory signaling by preventing their binding to HLA-C. Lirilumab, the most advanced agent is being studied in hematological malignancies e.g. Acute Myeloid Leukemia (AML) as monotherapy and in combination with 5-azacytidine. Lirilumab is also being studied in separate combinations with lenalidomide, elotuzumab and nivolumab in Multiple Myeloma (MM), in CLL in combination with rituximab, and in NHL with nivolumab. Its combination with nivolumab is also under evaluation in Melanoma, Head and Neck Cancer, Ovarian Cancer, Gastric Cancer and HCC. A combination trial of lirilumab and ipilimumab in select advanced cancer patients (CRPC, NSCLC) has been completed and results are awaited. Lirilumab monotherapy had no DLTs in a dose-escalation trial up to 10 mg/kg, although updated results reported grade 3 toxicities even at low doses. Full KIR occupancy (> 95%) was sustained during > 4 weeks for dose-levels ≥ 0.3 mg/kg.

- Lirilumab is the most advanced anti-KIR agent and is being studied in a number of hematologic malignancies, primarily in combinations.
- Engagement of KIR by HLA molecules results in inhibitory signaling that reduces NK cell-mediated killing and ADCC; anti-KIR agents such as lirilumab may improve the therapeutic efficacy of mAbs such as elotuzumab and rituximab that rely on NK cell mediated ADCC for their mechanism of action.
- Combinations with lirilumab need to be selected judiciously since it has demonstrated severe toxicities even at low doses as a single-agent.

2. Development of antibodies that target other inhibitory molecules such as CD47 and CEACAM-1/6 has been slow; early data in NSCLC.

Antibodies targeting CD47 and CEACAM-1/6 are under early clinical evaluation across solid tumors and haematological malignancies.

**CD47**: The SIRPα (signal regulatory protein α)-CD47 system is part of a ‘don’t-eat me’ signal sent out by tumor cells to avoid phagocytosis by macrophages. CD47 targeting antibodies have been proposed to promote macrophage phagocytosis of tumors. A recent report however has suggested that the therapeutic effects of CD47 targeting antibodies depend upon dendritic cell cross priming of CTLs and required the cytosolic DNA sensor STING. There are three agents targeting CD47 currently being evaluated. CC-90002 (Celgene) is currently in a Phase I trial that is recruiting patients with advanced solid and hematological malignancies. NI-1701 is a fully-human IgG molecule (Novimmune) with bispecific antigen binding regions, one targeting
CEACAM-1 and CEACAM-6: CEACAM-1 and CEACAM-6 are members of the carcinoembryonic antigen (CEA) family of immunoglobulin glycoprotein cell adhesion molecules (CAM) comprising at least 12 members. CEACAM-1 is present on tumor epithelial cells as well as on T-cells and undergoes trans-oligomerization resulting from homophilic interactions of its amino terminal IgV-like domains.

CM-24, a humanized immune-modulating antibody that binds CEACAM1 is being developed by cCAM Biotherapeutics which was recently acquired by Merck. A Phase I trial with CM-24 has been approved by the FDA and will be a first-in-human, open-label, multicenter, dose escalation study in cancer patients with select advanced or recurrent malignancies, including Melanoma, NSCLC, bladder, gastric, colorectal or ovarian cancer. CM-24’s effect is elicited by its ability to block the binding of CEACAM-1 on cancer cells to CEACAM-1 on certain immune cells. This abrogates the immunosuppressive function of CEACAM-1, promoting cell killing by T cells and NK cells. The effect of CEACAM-1 blockade does not lead to general immune activation, but to anticancer-specific activation.

CEACAM-6 is a glycosylated 90 kDa GPI-linked membrane adhesion molecule that forms homotypic and heterotypic bonds with CEACAM-1, 5 and -8. CEACAM-6 is expressed at low levels in normal epithelial, endothelial and hematopoietic cells including granulocytes, T-cells and NK cells. Binding and crosslinking of CEACAM-6 by cytotoxic T cells inhibits their activation and results in T-cell unresponsiveness. Blocking of CEACAM-6 on the surface of myeloma cells by specific monoclonal antibodies or CEACAM-6 gene knockdown by short interfering RNA restored T-cell reactivity against malignant plasma cells. An antibody conjugate agent (L-DOS47) that links urease to a single domain antibody AFAIKL2, that targets CEACAM6, is currently in clinical development by Helix Biopharma. Interim analysis of a monotherapy study of L-DOS47 in NSCLC has shown that 50% of patients had a radiological assessment of stable disease. Two patients completed six dosing cycles before discontinuing therapy. None of the patients treated to date have had a partial or complete response as defined by RECIST v1.1 definition. L-DOS47 was well tolerated for all patients treated within all cohorts. None of the treatment-related adverse events reported to date have met the definition of a dose-limiting toxicity.

• CD47 as an antitumor target needs further validation. It is not clear whether antitumor effects of CD47 targeting are indeed due to blocking its interactions with SIRPa and phagocytic clearance by macrophages.
  - CD47 is known to interact with other proteins such as integrins and thrombospondin-1
  - Anti-CD47 antibodies are also likely to have Fc-mediated ADCC activity
• Development of anti-CD47 should take into account potential on-target toxicities associated with inhibition of this pathway such as depletion of red blood cells.

Cd19 on B-cell tumors, and another that has CD47 blocking activity to disrupt the "don’t eat me" signal. Hu5F9-G4 (Stanford), another anti-CD47 antibody has shown positive results in several different models of childhood cancers and is currently in a Phase I trial in adult patients.
Strategies to deal with toxicities associated with 4-1BB agonists include careful titration and dosing, and combining with checkpoint inhibitors to strike a balance between stimulation and inhibition.

3. Agonistic antibodies targeting co-stimulatory receptors are beginning to show efficacy primarily in hematological malignancies and certain solid tumors such as Melanoma: In this category preliminary efficacy data is now becoming available with agents targeting CD137 (4-1BB), CD27 and CD40

**CD-137 (4-1BB):** CD-137 is a co-stimulatory receptor that is induced in CD4+ and CD8+ T-cells as well as NK cells, and other immune/ non-immune cells. It is constitutively expressed in Tregs as well. Ligation of CD137 with its ligand (CD137L/TNFSF9) results in activation of T-cells and acquisition of effector function, and ligation of CD137 on Tregs limits their suppressive function. Currently, there are two antibodies with early clinical data. Bristol-Myers Squibb had started development of BMS-663513 (urelumab) a specific anti-4-1BB agonist antibody (isotype IgG4). In a Phase I/II study with Melanoma, RCC and Ovarian Cancer, partial responses were limited to 6% of the Melanoma patients, although 17% of Melanoma patients and 14% of RCC patients had stable disease at 6 months or longer (41). Toxicity issues such as hepatitis were reported in a Phase II trial of metastatic Melanoma in 2011 leading to a termination of three other trials with urelumab. Development has recently restarted using urelumab at a lower dose in combination with other agents - in NHL with rituximab, in advanced/metastatic Colorectal and Head and Neck cancers with cetuximab and in Multiple Myeloma (MM) with elotuzumab. Pfizer’s PF-05082566 is the other agonist anti-4-1BB antibody (IgG2 isotype) with some clinical data. Data from a Phase I study that evaluated this agent in combination with rituximab in patients with relapsed or refractory NHL demonstrated antitumor activity. The ORR was reported to be 21%, and in rituximab-refractory patients the ORR was 29% (4/14). There were two CR with duration of response > 2 years and 2 PR (FL and MCL). The PFS for all of the rituximab-refractory responders was > 6 mo. In a second Phase I study of PF-05082566 in patients with advanced cancers, 27 patients have been treated with 22% (6/27) patients showing stable disease.

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**CD27:** CD27 (TNFRSF7) another co-stimulatory receptor that belongs to the TNFR superfamily is expressed constitutively on naive and effector T-cell subsets. Its ligand, CD70, is expressed on APCs. Paradoxically, it is also found on a number of tumor cells at very high levels. It is thought, however, that inhibitory signalling through the PD-1/PD-L1 axis is dominant over the stimulatory signalling from the CD27/CD70 axis. Varilumab, a CD27 agonist antibody from Celldex Therapeutics has completed a Phase I dose-escalation study, demonstrating potent immunologic activity consistent with its mechanism of action and antitumor activity in patients with

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**Clinical Development Summary: Key MoAs Targeting Co-Stimulatory Pathways**

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advanced, refractory disease. Nineteen patients [3 HL, 5 Follicular Lymphoma (FL), 9 DLBCL, 2 unspecified NHL] have received the drug. A patient with Stage IV HL who had previously failed chemotherapy, autologous stem cell transplant, and brentuximab vedotin experienced a complete response (CR) and remains in remission at 8.6+ months. Three additional patients had stable disease (4.5, 5.6 and 14.0 months), including a patient with FL who has completed 3 cycles of therapy and a patient with marginal zone lymphoma (MZL) with 36% shrinkage in measurable disease.

CD40: Unlike the other TNFRSF members described here CD40 (TNFRSF5) is predominantly expressed constitutively on APCs, as well as on B-cells and a number of non-hematopoietic cells. The ligand CD40L on the other hand is expressed on T cells, B cells and NK cells. CD40 ligation induces APC maturation and production of cytokines which is essential for T-cell activation. Currently there are four CD40 agonist agents in development: CP-870,893/RO700978 (Roche), ADC-1013 (Alligator Bioscience AB), SEA-CD40 (Seattle Genetics), APX005M (Apexigen) with one discontinued (SGN-40, dacetuzumab). In addition, lucatumumab (HCD122, Novartis) is a fully humanized anti-CD40 antibody that blocks interaction of CD40L with CD40 and also mediates ADCC. Data is available from a monotherapy trial with CP-870,893 in patients with advanced solid tumors. The most common adverse event was cytokine release syndrome (grade 1 to 2) which included chills, rigors, and fever. Four patients with Melanoma (14% of all patients and 27% of Melanoma patients) had objective partial responses at restaging (day 43). Another new Phase I trial finds that the combination of the antibody CP-870,893, and the checkpoint inhibitor tremelimunumab is safe and effective in patients with Melanoma. Tumors shrank or disappeared in 27% of patients and the combination was also associated with two complete responses (9%) at the MTD, four partial responses (18%), and two patients with stable disease. Median progression-free survival was 3.2 months, and overall survival was 26.1 months. The toxicity for the combination was the same as for each drug as a single agent, and the side effects were non-overlapping. Lucatumumab has also completed Phase I trials in MM and B-cell CLL. In the lucatumumab CLL trial 26 patients were enrolled of which 17 had stable disease (mean duration of 76 days, range 29-504 days) and one patient had a nodular partial response for 230 days.

GITR: GITR was first identified as a constitutively expressed receptor on Tregs. GITR is also inducibly regulated in CD4+ and CD8+ cells upon activation and maintains expression for several days. Interestingly GITR agonism increases expansion of thymic as well as peripheral T-cells, apart from its stimulation of effector T-cell proliferation. The preclinical evaluation of GITR antibodies revealed some very strong data, including in combination settings with anti-PD-1 agents and CTLA4 blockade in which anti-GITR antibodies essentially synergized with these agents to eliminate established tumors. GITR agonism is also synergistic with chemotheraphy in preclinical models. Clinical development of GITR antibodies has been slow. TRX518 is an antibody from GITR, Inc., that is currently recruiting for a Phase I trial in advanced Melanoma and other solid tumors. Merck is also advancing a Phase I study with the anti-GITR antibody MK-4166.

Clinical efficacy with agonistic GITR antibodies is yet to be evidenced; it remains to be seen whether activation of GITR signaling in effector T cells by these agents can overcome the proliferation and accumulation of Treg cells in tumor tissue, also simultaneously induced through GITR.

OX-40: OX-40 is induced transiently following T-cell activation and is expressed on CD4+ and CD8+ T cells, and also on NKT cells, NK cells and neutrophils. An agonistic murine antibody targeting the extracellular domain of OX-40 (9B12, AgonOx) was used in a Phase I clinical trial in patients with advanced cancer. Patients treated with one course of the mAb showed an acceptable toxicity profile and regression of at least one metastatic lesion in 12 of 30 patients. Mechanistically, this treatment increased T and B cell responses to reporter antigen immunizations (e.g. KLH), led to preferential upregulation of OX40 on CD4+FoxP3+ regulatory T cells in tumor infiltrating lymphocytes, and increased the antitumor reactivity of T and B cells in patients with Melanoma. The AgonOx program was acquired by Medimmune which took the 9B12 mAb (renamed MEDI6469) and developed a humanized version MEDI0562. A Phase I, multicenter, open-label study with this agent in adults with select solid tumors is currently recruiting patients. Other agents in clinical development that target OX-40 include RG7888 (MOXR0916, Roche/Genentech) and anti-OX-40 mAb acquired from UTMDACC by GSK.
4. Small molecule immuno-oncology agents that block tryptophan catabolism have demonstrated efficacy in early trials in Melanoma, NSCLC, Breast Cancer and CRC

IDO: Indoleamine 2,3-dioxygenase 1 (IDO1) is a tryptophan-catabolizing enzyme that is overexpressed in many cancers which induces immune tolerance by suppressing T-cell responses. The agent that is farthest along in the clinic appears to be INC024360 (epacadostat, Incyte). Initial data from a dose-escalation study in patients with unresectable metastatic Melanoma suggested increased anti-tumor efficacy when epacadostat was administered in combination with ipilimumab. Updated results from this study which involved 40 patients with Metastatic Melanoma (30 who were immunotherapy naive and 10 who were previously given immunotherapy) have recently become available. Among the 30 immunotherapy-naïve patients, ORR was 30% (9/30) per immune related response criteria (irRC) and 27% (8/30) per RECIST, and CR rate per both criteria was 10%. At data cutoff, responses were ongoing in 7 patients; these patients were progression-free with at least 6 months of follow up. The disease control rate (DCR; CR+PR+SD) was 60% per irRC and 57% per RECIST. Among 10 patients with a best response of SD by irRC or RECIST, 5 were...
progression-free by either criteria with at least 6 months of follow up. Median PFS was 8.3 months by irRC and 6.7 months by RECIST. Among 10 patients previously treated with immunotherapy, the DCR by both criteria was 30% (all Sds). Epacadostat has also demonstrated early efficacy as monotherapy in CRC patients.

5. Small molecules targeting chemokine signalling have demonstrated early efficacy data in Breast Cancer, Bladder Cancer and Squamous Cell Cancer

A variety of immune cells home to tumor sites depending upon the chemokine ligands in the microenvironment and the receptors expressed on the infiltrating cells. The sum total of these effects determines whether the microenvironment is immune suppressing or responsive to immunotherapies. The human genome contains over 44 chemokine members. Chemokines are structurally divided into 4 subgroups, namely, CXC, CC, CX3C, and C. The first 2 cysteines are separated by 1 and 3 amino acids in CXC and CX3C chemokines respectively, while the first 2 cysteines are adjacent in CC chemokines. The L or R in the nomenclature denotes ligand or receptor respectively. Chemokines such as CCL2, CCL5, CCL7, CCL8, and CXCL12 and macrophage colony stimulating factor (M-CSF) attract macrophages (TAMs) derived mostly from circulating monocytes. The following chemokine MoAs have been explored clinically for cancer treatment.

CXCR 1/2: A number of CXCR1 and/or CXCR2 small molecule inhibitors are under development although most of these antagonists have only been used in clinical trials for the treatment of inflammatory conditions. For example, reparixin, an inhibitor of both CXCL8 receptors CXCR1 and CXCR2, has been shown to attenuate inflammatory responses in various models. Efficacy of reparixin with paclitaxel combination was demonstrated both in hormone receptor positive breast cancer and triple receptor negative disease. To date, 5 confirmed responses (2 CR, 3 PR) have been recorded among 18 patients who underwent at least 1 tumor assessment (at 8 weeks). Response duration was 20m+ and 3m+(for CR) and 9m+, 6m+, 2m+ (for PR). Another CXCR2 antagonist, AZD5069, was shown to be well tolerated in a Phase I study in healthy subjects and a number of Phase II studies have been initiated with this agent.

CXCR4: CXCR4 is another chemokine receptor whose inhibition is being explored in the clinic. Activation of CXCR4 through its ligand CXCL12 contributes to metastasis in several types of tumors, where circulating epithelial tumor cells express CXCR4 and common metastasis sites express abundant ligand, and ligand/receptor interaction has been shown to

The other agent that has moved along in the clinic is indoximod (1-methyl-D-tryptophan, Newlink Genetics). Results from a combination trial of this agent with ipilimumab in Melanoma have also become available. This was a Phase Ib study of indoximod dose escalation in combination with ipilimumab (3mg/kg q3 weeks x 4 doses) in patients with unresectable Stage III or IV Melanoma. As of April 26, 2015, 7 patients remain on study (2 discontinued due to progression of disease) and no dose limiting toxicities (DLTs) were observed. Indoximod in combination with docetaxel has also demonstrated PR in patients with Breast Cancer and NSCLC. A third IDO inhibitor that has been moving along the clinical development path is GDC 0919. In a Phase Ia study of the safety, pharmacokinetics, and pharmacodynamics of GDC-0919 in patients with recurrent/advanced solid tumors 19 patients were treated and 3 remained active (16 patients discontinued treatment due to progressive disease). Best RECIST responses among 16 patients with on-treatment tumor assessments were 7 (44%) stable disease (SD, lasting ≥4 cycles in 4 patients) and 9 (56%) progressive disease. irRC and PD marker data are pending.

Among the newer immune-oncology agents IDO inhibitor epacadostat (INCB024360) has been one of the most actively investigated and is currently in combination trials with a number of checkpoint blockers including pembrolizumab, nivolumab, MEDI4736, MPDL3280A and ipilimumab.

While generally regarded as a ‘safe’ immunomodulator, the dose-limiting toxicity of epacadostat in combination with ipilimumab was noted to be liver damage as measured by transaminase elevation.

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CXCR 1/2: A number of CXCR1 and/or CXCR2 small molecule inhibitors are under development although most of these antagonists have only been used in clinical trials for the treatment of inflammatory conditions. For example, reparixin, an inhibitor of both CXCL8 receptors CXCR1 and CXCR2, has been shown to attenuate inflammatory responses in various models. Efficacy of reparixin with paclitaxel combination was demonstrated both in hormone receptor positive breast cancer and triple receptor negative disease. To date, 5 confirmed responses (2 CR, 3 PR) have been recorded among 18 patients who underwent at least 1 tumor assessment (at 8 weeks). Response duration was 20m+ and 3m+(for CR) and 9m+, 6m+, 2m+ (for PR). Another CXCR2 antagonist, AZD5069, was shown to be well tolerated in a Phase I study in healthy subjects and a number of Phase II studies have been initiated with this agent.

CXCR4: CXCR4 is another chemokine receptor whose inhibition is being explored in the clinic. Activation of CXCR4 through its ligand CXCL12 contributes to metastasis in several types of tumors, where circulating epithelial tumor cells express CXCR4 and common metastasis sites express abundant ligand, and ligand/receptor interaction has been shown to
promote metastasis. The agents being developed include BL8040, LY2510924, Plerixafor, and PTX-9908. BL-8040 binds to CXCR4 on tumor cells and blocks its availability to the ligand CXCL12 leading to the release of tumor cells from the protective microenvironment of the bone marrow resulting in chemosensitization. BL-8040 is currently undergoing a Phase Ila clinical trial for the treatment of relapsed or refractory AML. Positive data from this study was recently reported showing substantial mobilization of AML cancer cells from the bone marrow to the peripheral blood and robust apoptosis of these cells, as well as an excellent safety and tolerability profile. BL-8040 is also scheduled to commence other trials including one for consolidation treatment for AML patients who have undergone induction chemotherapy, a trial to improve the response of FLT3-ITD mutated AML patients to treatment with sorafenib, and another trial for the treatment of hypoplastic myelodysplastic syndrome (hMDS) a subtype of myelodysplastic syndrome and aplastic anemia (AA). The CXCR4 peptide antagonist LY2510924 is also undergoing a Phase I trial in patients with advanced cancer, where the best response was stable disease in 20% patients. Data from a randomized Phase II study of sunitinib + CXCR4 inhibitor LY2510924 versus sunitinib alone in first-line treatment of patients with metastatic renal cell carcinoma did not reveal any improvement in efficacy over single agent sunitinib.

CSF1R: Tumors often overexpress CSF1 which recruits TAMs and MDSCs resulting in the promotion of a Th2-type polarized environment with high levels of CD4+ T cells, and low levels of CD8+ cytotoxic T cells. Several CSF1R inhibitors are undergoing clinical development including PLX3397, JNJ-40346527, FPAA08, AC-708 and IMC-CS4. PLX3397 in addition to inhibiting CSF1R is also potent and selective inhibitor of, KIT and FLT3-ITD. In trials where patients with advanced solid tumors were treated with PLX3397 doses of 100-900 mg daily there was pronounced reduction in a defined subset of circulating monocytes (CD14dim/CD16+) as well as circulating tumor cells (CTCs). In another Phase Ib study of PLX3397 combined with paclitaxel in patients with advanced solid tumors, of 23 efficacy evaluable patients, 4 had PR (2 breast, 1 squamous cell, 1 bladder cancer), 10 had SD, 9 had PD. In all patients plasma CSF-1 levels increased and CD14dim/CD16+ monocyte levels decreased, indicating blockade of CSF1R signalling. A Phase I/II study of JNJ-40346527, in relapsed or refractory classical Hodgkin lymphoma (cHL) enrolled 21 patients, which demonstrated complete remission in 1 patient (duration, +352 days) and stable disease in 11 patients: (duration, 1.5–8 months).

6. TLR agents have demonstrated early single agent data in Melanoma, Ovarian Cancer, CRC and also in early CTCL

TLR 7/8/9: TLRs play a key role in immunity through recognition of microbial pathogen associated molecular patterns (PAMPs) and endogenous danger signals (DAMPs) released from dying/stressed cells. There are at least 10 subtypes expressed in humans which could mediate either pro- or anti-tumor effects depending on their cellular location, type of TLR activated and downstream signalling following their activation. TLR expression is primarily restricted to monocytes, macrophages and DCs although they are also found on other cell types. They can also be inducibly expressed on certain tumor and T cells. The protumorigenic roles of TLR are through activation of downstream MAPK, JNK, p38 and ERK signaling pathways while antitumor effects are mediated via activation of both the adaptive and innate arms of the immune system. In clinical trials, TLR agonists, particularly those of TLRs 7, 8 and 9 are under evaluation as adjuvants to anticancer vaccines and immunotherapies to stimulate greater immune responses.

There are seven agonistic agents targeting members of the TLR family that are in early stages of clinical development. TLR8 agonists stimulate myeloid dendritic cells, monocytes, and NK cells. In a prospective Phase Ib clinical trial of neoadjuvant cetuximab and motolimod (VTX-2337, VentiRx) in patients with Head and Neck Cancer, the combination was well tolerated. Partial responses and stable disease were observed in some patients. In Ovarian Cancer, treatment with pegylated doxorubicin (PLD) +
Conclusion:

We have highlighted key novel antibody-based and small molecule immuno-oncology agents that are currently in clinical development. The overall goal of these newer immunotherapies is to improve upon the recent advances in the field by inducing durable responses in a larger number of patients as compared to what is now achieved with PD-1 or CTLA-4 blockade monotherapies. Although initially approved for Melanoma, the greatest impact of anti-PD-1/PD-L1 therapies is perhaps realized in NSCLC - a tumor which was historically considered to be non-immunoresponsive. Irrespective of histology, the benefit of these agents is seen across patient segments in NSCLC. These agents have also shown very good efficacy in relapsed metastatic RCC, where traditionally few options have existed. However, several tumor types continue to be non-responsive to PD-1/PD-L1 blockade monotherapies including multiple myeloma and a large fraction of CRC patients. The PD-1 pathway is just one of many potential inhibitory systems that tumors use to anergize T cells. Clearly further work is needed to precisely define which pathway is operative in which cancer. It is likely that many of these patients will respond when some of the newer agents described here are used in combination with current SoCs, other emerging agents or PD-1/PD-L1 blockers. The successful development of many of these combinations is dependent upon improved understanding of the immune mechanisms of these newer agents coupled with the use of appropriate predictive biomarker assays to select the right patient populations. The next few years should reveal whether the hype surrounding immunotherapies was justified and whether the hope of long-lasting remissions is being realized for more patients.

VTX-2337 showed early efficacy with 1 partial response (13%) and 63% patients had stable disease. VTX-2337 with PLD has fast track status in Ovarian Cancer.\(^{75, 76}\)

- TLR agonists have been disappointing in the clinic as single agents suggesting that they need to be employed in combinations to enhance their immunostimulatory properties.
- Multi TLR agonist approaches such as that employed with BCG (which stimulates TLRs 2, 3, 4 and possibly 9) in urinary bladder cancer have been the most successful.
- Combining TLR agonists with agents that can activate dendritic cells such as anti-CD40 agonist antibodies can have synergistic potential.

TLR9 agonist PF-3512676 (Pfizer) demonstrated durable partial responses and stable disease in a Phase II trial of metastatic Melanoma. Activity was also seen in a Phase I dose-escalating trial in patients with CTCL (N=28) with 3 CR and 6 PR. However no responses were seen in CLL and while late clinical responses were observed in NHL. In a Phase III trial, combination with chemotherapy in NSCLC did not improve responses over monotherapy and clinical development was terminated due to severe toxicities.\(^{77}\) In the Phase II IMPACT trial, clinical efficacy, safety, and immunological effects of another TLR9 agonist MGN1703 were assessed as switch maintenance after first line induction therapy in mCRC: improved efficacy with MGN1703 compared to placebo was observed with an HR of 0.56 for PFS on maintenance (p=0.070). Pretreatment characteristics which were predictive of a PFS benefit - normalized CEA, objective response, and the presence of activated NKT cells - were also associated with OS benefit in exploratory analysis. Other agents in early stage development include SD101 (Dynavax), MIS416 (Innate Pharma) and resiquimod (3M Pharma/Spirig Pharma).\(^{78, 79}\)


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