Immunomodulatory antibodies for the treatment of lymphoma: Report on the CALYM Workshop

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ABSTRACT
In November 2015, the CALYM Carnot Institute held a 2-d workshop to discuss the current and future development of immunomodulatory antibodies for the treatment of lymphoma. Highlights from the workshop are presented in this article.

ABREVIATIONS: BCR, B-cell receptor; CALYM, Consortium for the Acceleration of innovation and its transfer to the field of Lymphoma; CHLs, primary classical HLs; CTLA-4, anti-cytotoxic T-lymphocyte-associated antigen 4; CXL12, chemokine ligand 12; DC-SIGN, dendritic cell-specific ICAM-3-grabbing non-integrin; DLBCL, diffuse large B-cell lymphoma; FcγRIV, Fcγ-receptor IV; FL, follicular lymphoma; GITR, anti-glucocorticoid-induced TNFR-related protein; GOELAMS, Groupe Ouest-Est des Leucemies et Autres Maladies du Sang; HL, Hodgkin lymphoma; HVEM, herpes virus entry mediator; Ig, immunoglobulin; IL, interleukin; irAEs, immune-related adverse events; LG3, lymphocyte-activation gene 3; MAPK, mitogen-activated protein kinase; MEK, Mitogen-activate protein kinase kinase; MLBCL, mediastinal large B-cell lymphoma; NK, natural killer; PD-1, programmed cell death 1 receptor; PD-L1, programmed cell death receptor ligand 1; RS, Reed–Sternberg; SHP2, Src-homology 2 domain-containing phosphatase 2; sPD-L1, soluble PD-L1; T eff, the effector T cells; TILs, tumor-infiltrating lymphocytes; TIM3, T-cell Ig and mucin domain containing 3; TLR9, Toll-like receptor 9; Tregs, regulatory T cells.

Foreword
Highlights from Day 1 of the workshop are presented in this article. Sadly, Day 2 of the workshop was cancelled as a result of the tragic events that unfolded in Paris on the evening of Friday 13th November 2015. We are grateful, however, that a number of the intended presenters have provided summaries of their presentations, excerpts from which are included. The CALYM Carnot Institute would like to acknowledge and thank all of the workshop contributors for their attendance and participation. The Institute also wishes to express their sympathy and solidarity with the people of France, and Paris in particular.

Workshop highlights – Day 1
Introduction
The CALYM (Consortium for the Acceleration of innovation and its transfer to the field of Lymphoma) Workshop was planned for 13th and 14th November 2015 in Paris, France, and was designed to bring together people from industry and the academic world to discuss the current and future development of immunomodulatory antibodies for the treatment of lymphoma. The 2-d program was planned to include contributions from 17 experts in the field of cancer or immunomodulation.
**Basic cancer immunology**

Following the welcome address by Gilles Salles, Philippe Gaulard introduced the CALYM Carnot Institute and outlined its mandate before introducing the first session, *Basic cancer immunology*. Robert Schreiber explained that the immune system protects against cancer development and shapes cancer immunogenicity via cancer immunoediting. He defined the three phases of immunoediting as elimination, equilibrium, and escape. Highly antigenic, tumor-specific antigens are favored targets of immunoediting. Mutant “neoantigens” remaining in tumors after immunoediting are targets of T cells that are reinvigorated by checkpoint-blockade.

Dr Schreiber continued by focusing on the role of programmed cell death 1 receptor (PD-1) ligand 1 (PD-L1) in cancer-induced immunosuppression. PD-L1 expression on immunoedited, progressively growing tumor cells plays an important role in mediating tumor escape from immune control. However, physiologically-induced PD-L1 expression on highly immunogenic unedited tumor cells is not sufficient to counteract immune effector mechanisms driven by “strong” tumor antigens. Adaptive immune resistance occurs only after cancer immunoediting has eliminated tumor cells expressing the “strongest” tumor antigens.

**Lessons learned from solid tumors**

In the second session, *Lessons learned from solid tumors*, Ira Mellman discussed the mechanistic basis of cancer immunotherapy and lessons learned from the study of solid tumors. He explained that, although a wide range of tumor types respond to anti-PD-1 or -PD-L1 treatments, only a subset of patients benefit (overall response rate is typically 10–30%). Dr Mellman explained how PD-L1 expression pattern can predict response to anti-PD-L1 monotherapy. For example, in patients with bladder cancer, clinical response is better in patients whose immune infiltrates express PD-L1 versus those who are PD-L1-negative patients with lung cancer are more likely to respond if they express PD-L1 on their tumor cells, or both on tumor cells and immune cell infiltrates.

Using a liposome-based Förster resonance energy transfer quenching assay and other in vitro assays, Dr Mellman has found that PD-1 may preferentially regulate T cell activity via inactivation of CD28 and that the protein tyrosine phosphatase Shp2 and PD-1 preferentially dephosphorylate CD28 over CD3ζ. Dr Mellman used in vitro and mouse studies to show that mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitors potentiate T-cell based antitumor immunity and can combine successfully with anti-PD-L1 immunotherapy. He explained that an active MAPK pathway may only be required for naïve T cell expansion and differentiation into memory cells. He went on to discuss the potential of vaccines when there is insufficient T-cell immunogenicity.

Olivier Lambotte discussed the side effects of anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and anti-PD-L1 treatments, outlining that differences in their safety profiles are attributable to differences in their mechanisms of action and the expression profiles of their targets. He highlighted data to show that these drugs are associated with immune-related adverse events (irAEs). Frequency of irAEs varies with the type of cancer and is generally less severe than those secondary to standard chemotherapy. irAEs with ipilimumab are more common than those with anti-PD-1 or -PD-L1 treatments. Dr Lambotte underlined that it is important to understand how irAEs can be predicted, how they should be managed and potential long-term risks from immunosuppression.

In the following presentation, Pierre Coulie described the immunological requirements for clinical responses to immunostimulatory antibodies, explaining that requirements include antigenicity (e.g., mutated or overexpressed antigens recognized by T cells), spontaneous immunogenicity (T cell responses before treatment), activity of the mechanism(s) targeted by the antibodies, and absence of tumor resistance or major immunosuppressive activity at the tumor site(s). Dr Coulie has investigated the spontaneous immunogenicity of CD8-positive T cells toward untreated primary human breast cancers and found that some tumors are spontaneously immunogenic. He showed that CD8-positive tumor-infiltrating lymphocytes (TILs) in primary breast cancers are oligoclonal and that TIL oligoclonality is not necessarily a sign of ongoing antitumour T cell responses. [Personal communication] Some primary breast cancers contain tumor-specific CD8-positive TILs at frequencies similar to those found in melanoma.

In the penultimate presentation of the session, Thierry Fest provided insights into soluble PD-L1 (sPD-L1). PD-L1 may be overexpressed by tumor cells in Hodgkin and non-Hodgkin lymphomas, including diffuse large B-cell lymphomas (DLBCL). This overexpression may result from several mechanisms including cyto genetic alterations (amplification or rearrangement) involving the PD-L1 locus. In the French multicentre GOELAMS (Groupe Ouest-Est des Leucemies et Autres Maladies du Sang) 075 trial in patients with DLBCL, a significant increase of sPD-L1 was observed in patients’ plasma compared with age- and gender-matched normal controls. In most cases sPD-L1 levels returned to normal after complete disease remission. These results were consistent with another patient cohort from Australia, the Iowa/Mayo Spore cohort in the United States and two cohorts from France. A higher level of sPD-L1 was associated with a lower overall survival in the GOELAMS 075 trial. Dr Fest explained that DLBCL tumors have a different CD4-positive:CD8-positive T cell ratio compared with other lymphomas, and that sPD-L1 is particularly expressed in monocytes and myeloid cells in patients with DLBCL. Patients with Hodgkin lymphoma (HL) express high sPD-L1 levels although PD-L1 levels were found not associated with HL patient outcomes.

The final presentation was given by Alan Korman who discussed the development of immunomodulatory antibodies and data obtained with an anti-glucocorticoid-induced TNFR-related protein (GITR) antibody. He showed preclinical data of anti-PD-1 and anti-CTLA-4 antibodies in the MC38 tumor model, and described the mechanism of CTLA-4/PD-1 blockade. Depletion of regulatory T cells (Tregs) by anti-CTLA-4 antibodies and CTLA-4 blockade activates CD4- and CD8-positive T cells, whereas anti-PD-1 antibodies activate CD8-positive T cells leading to a modest increase in CD8-positive T cell numbers. Unresolved questions remain surrounding the functional effect of combined CTLA-4/PD-1 blockade in the...
remaining Tregs, activity of dual blockade within the same cell, and whether CTLA-4/PD-1 double-positive CD8-positive TILs in the MC38 model are tumor antigen specific. Dr Korman showed that agonistic anti-GITR antibodies can promote anti-tumor activity in the MC38 model, deplete tumor Tregs and promote interleukin (IL)-2 production. In the MC38 tumor model, antitumor activity of the anti-mouse GITR antibody DTA-1 is potentiated by an anti-PD-1 monoclonal antibody.

**Immunomodulatory antibodies in lymphoma**

Ron Levy opened the third session, “Immunomodulatory antibodies in lymphoma” by providing a historical framework of immunotherapy in lymphoma. He introduced a timeline of progress in technologies and treatments in the past 100 y, including the “magic bullet concept” in 1897, development of hybridoma technology in the 1970s, and approval of rituximab in 1997.28 Dr Levy highlighted important points about PD-1 blockade, including why only some patients and tumor types respond, the need to understand “pseudoprogression” (tumor flare vs. immune cell infiltration) and how best to increase response rates—be this via enhancement of PD-L1 expression or the use of combination therapy. He showed how the combination of an anti-PD-L1 antibody and ibrutinib, a Bruton’s tyrosine kinase inhibitor, synergistically reduced tumor volume and increased survival in mouse models of B-cell lymphoma and colon cancer.27 He explained that the combination of ibrutinib and PD-L1 inhibitors is under investigation in clinical trials (NCT02403271).

Margaret Shipp discussed lymphoma biology and the cancer microenvironment in relation to immunomodulatory targets,28 hypothesizing that lymphoid malignancies most likely to respond to immunomodulation are those with evidence of innate and/or adaptive antitumour immune responses. Dr Shipp explained that primary classical HLs (cHLs) contain only small numbers of malignant Reed–Sternberg (RS) cells but that they do contain extensive inflammatory/immune cell infiltrates.29 The HL RS cells are known to interact with tumor-infiltrating T cells (e.g., CD8-positive T cells and CD4-positive T helper and Treg cells).30 Dr Shipp explained that CHL and mediastinal large B-cell lymphoma (MLBCL) have overlapping genetic, clinical, and immunological features—including chromosome 9p24.1 copy number alterations leading to PD-L1 overexpression—that support recent hypotheses suggesting a pathogenic relationship between these two diseases.12,31–38 The genetic basis for enhanced PD-1 signaling likely explains the therapeutic efficacy of PD-1 blockade in these diseases.12,32,39 She also showed that PD-L1 is expressed in Epstein–Barr virus-associated large B-cell lymphoma subtypes, and suggested that such tumors may also respond to PD-1/PD-L1 blockade.40

Following on from Dr Shipp, Karin Tarte discussed the tumor-supportive folliclar lymphoma (FL) cell niche, showing that recognition of FL has shifted from a B-cell autonomous disease to a niche-based disease. Dr Tarte explained that two key features of the FL microenvironment are a high level of IL-4 expression and presence of a follicular helper T cell (TFH) gene signature. Studies suggest that FLs have a specific infiltration by PD-1-positive follicular Tregs and TFH, and show that the TFHs in FL are activated and are the main producers of IL-4 in the FL cell niche.41–44 Dr Tarte also showed that IL-4-treated lymphoid stromal cells upregulate expression of chemokine (C-X-C motif) ligand 12 (CXCL12) in vitro, IL-4 increases CXCL12 expression in mice and CXCL12 is involved in stroma-dependant B-cell migration and adhesion.45 Regarding FL tumor-associated macrophages, she suggested that FL-stromal cells produce large amounts of the monocyte-recruiting chemokine CCL2; IL-4 upregulates the mannose-binding lectin dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) on macrophages; and macrophage-derived DC-SIGN is able to trigger antigen-independent activation of FL IgM BCR that is specifically highly mannosylated on its variable regions.46 She concluded that a better understanding of the stromal crosstalk network can potentially provide numerous therapeutic targets in FL.

Holbrook Kohrt began his presentation by discussing immunomodulation of innate immune cells in lymphoma, which he puts forward as a new frontier in cancer treatment. He described 4-1BB (CD137) as an inducible co-stimulatory target on natural killer (NK) cells and showed how an agonistic anti-CD137 antibody can enhance the in vivo antitumour activity of tumor-targeting monoclonal antibodies, such as rituximab (an antibody against CD20),47 trastuzumab (an antibody against human epidermal growth factor receptor 2)48 or cetuximab (an antibody against epidermal growth factor receptor).49 He discussed Phase I clinical trials with anti-CD137 antibodies (urelumab or PF#x2011;05082566) given in combination with rituximab (NCT01775631, NCT01307267) or cetuximab (NCT02110082), and a Phase I study of elotuzumab (an antibody against signaling lymphocyte activation molecule family member 7) in combination with either urelumab or lirilumab (an antibody against killer-cell immunoglobulin-like receptors) (NCT02252263). Following treatment in a clinical setting, urelumab can enhance CD4- and CD8-positive T cell responses and increase the proportion of CD56-positive NK cells. He summarized that, to increase innate immunity: antibody-dependent cell-mediated immunity can be enhanced using an anti-CD137 antibody, anti-KIR antibody or a γδ-T cell agonist (bromohydrin pyrophosphate); antigen presentation can be augmented using a Toll-like receptor 9 (TLR9) agonist; and phagocytosis can be enhanced using an anti-CD47 antibody.50

Aurélien Marabelle presented on the strategy of in situ immunomodulation, where immunostimulatory drugs are injected directly into tumors in order to prime locally a systemic antitumor immune response. Indeed, although immunomodulatory agents have shown efficacy in a number of different cancer types, major challenges remain: immune toxicity, cost, and how to overcome resistance to immune checkpoint blockade.51 Dr Marabelle stated that in situ immunomodulation should address these challenges, but explained that the concept is neither new nor widely-accepted, and relies upon injection of immunostimulatory drugs at a local tumor site to prime the body and create a systemic antitumour immune response including at non-injected tumor sites.

Dr Marabelle illustrated this concept by presenting both preclinical and clinical data where combinations of immunostimulatory products injected into tumors can generate tumor responses in non-injected tumor sites. Notably, he discussed data from a murine lymphoma model where mice were treated...
with an intra-tumoral triple combination of CpG oligonucleotide 7909 (a TLR9 agonist), an anti-OX40 antibody and an anti-CTLA-4 antibody. In a multiple tumor mouse model, this local therapy (administered at one site) was able to generate a T-cell mediated antitumor immune response able to eradicate tumors even at non-injected distant tumor sites, including in the brain. Dr Marabelle then presented the human translation of such an approach in patients with follicular B-cell and T-cell lymphoma. Such patients treated with local injections of CpG 7909, together with low dose 2 x 2 Gy flash irradiation at one tumor site, could also generate systemic antitumor immunity and tumor responses at non-injected tumor sites.

In the final presentation of Day 1, Stephen Ansell discussed preliminary results of clinical trials with immunomodulatory antibodies (ipilimumab, pidilizumab, nivolumab, pembrolizumab, varilimub, and dacetuzumab) in patients with lymphoma and other cancers. He addressed the rationale for using the antibodies, how they work, how well they work and whether they should be used alone or in combination. Dr Ansell explained that the “immune optimization approach” can be improved by inhibiting more than one checkpoint (e.g., PD-1/PD-L1 and CTLA-4/lymphocyte-activation gene 3 [LAG3]/T-cell Ig and mucin domain containing 3 [TIM3]), by simultaneously blocking an inhibitory and activating a stimulatory signal (e.g., PD-1/PD-L1 and 4-1BB/OX-40), or by using different immune activators. Dr Ansell outlined that optimizing immune function is the new therapeutic “frontier” in B-cell lymphomas and that immune checkpoint inhibitors hold real promise in HL and non-HL. Multiple new agents are in development (including anti-PD-L1, LAG3, and TIM3 antibodies), which can block immune suppression or induce immune stimulation. The next clinical challenge will be to appropriately and effectively incorporate these promising immunological agents into combination treatment approaches.

**Workshop highlights – Day 2**

**New perspectives in immunomodulation**

**Targeting immune regulation at the tumor site, Sergio A. Quezada and Frederick A. Vargas**

Within the tumor microenvironment, different components impede the immune response against cancer cells and are a critical obstacle to the success of cancer immunotherapy. Among these, the accumulation of Treg and the reduction of the effector T cells (Teff)/Treg ratio favor tumor progression and are associated with worse prognosis in several human cancers. Treg elimination in the tumor microenvironment can be achieved through antibodies that target molecules expressed preferentially in Treg and that induce antibody-mediated cell cytotoxicity, e.g., CTLA-4. Therapy with anti-CTLA-4 has been effective for cancer treatment in murine models and in humans. The efficacy of CTLA-4 depends on blockade of this target in Teff and in Treg. In mice, anti-CTLA-4 also depletes Treg in the tumor but not in peripheral lymphoid organs. This site specificity in mice is due to the expression of Fcγ-receptor IV (FcγRIV) in myeloid cells present in the tumor microenvironment, as well as high expression of the target in tumor-infiltrating Treg. However, the role of FcγRs in the effectiveness of anti-CTLA-4 therapy or therapies with other immunomodulatory antibodies in humans is not known.

Studies investigating the density of targets of clinically relevant immunomodulatory antibodies in different subpopulations of tumor-infiltrating immune cells and the expression of FcγR subtypes in murine tumor models have identified molecules that are preferentially expressed in Treg—and therefore are potential targets for depletion of these cells. Owing to inter-species differences in the repertoire and cellular distribution of FcγRs, this has also been studied in humanized-FcγR (huFcγR) mice. These were compared with samples of human melanoma and similar expression profiles of targets of immunomodulatory antibodies, including CTLA-4, were observed as well as infiltration of myeloid cells that express FcγRs. Using an antibody targeting mCTLA-4 with a human IgG1 isotype in huFcγR mice, efficient depletion of Treg was seen. Furthermore, mutation of the Fc region of the antibody to enhance its affinity to activating huFcγRs resulted in more effective selective Treg depletion in the tumor.

These findings are relevant for CTLA-4 and for other targets expressed preferentially on Treg, as has been shown with anti-GITR and anti-OX40 therapies. Only systematic characterization of the immunological landscape of human cancers will enable a greater understanding and optimization of therapies targeting immunomodulatory molecules through the development of antibodies with the optimal isotype (that depends on the target) and through optimizing combination therapies.

**Novel immune modulatory pathways for innate effectors and Treg, Daniel Olive**

Additional checkpoint pathways should be considered in malignant lymphomas, namely BTLA-HVEM and ICOS-ICOSL. BTLA, a member of the CD28:B7 family, is expressed by most lymphocytes and its ligand HVEM (herpes virus entry mediator) displays frequent abnormalities in human B cell malignancies, especially in FL. Blockade of BTLA-HVEM interaction has been shown to result in a significant increase in Vγ9Vδ2 T cell proliferation—Vγ9Vδ2 cells are a major peripheral blood T cell subset in humans displaying a broad reactivity against microbial agents and tumors. HVEM positive lymphoma cells have the potential to reduce the proliferation of intra-nodal Vγ9Vδ2 T cells in a BTLA-dependent manner. This suggests that HVEM dysregulation may play a role in lymphomagenesis by interacting with Vγ9Vδ2 T cell proliferation and BTLA stimulation of Vγ9Vδ2 T cells could be a new mechanism of immune escape by lymphoma cells.

**Choosing the right IgG subclass, Hervé Watier**

Murine experimental models have suggested that anti-CTLA-4 antibodies work better if they are based on a cytotoxic IgG subclass (IgG2a in mice) but ipilimumab (IgG1, possibly cytotoxic) and tremelimumab (IgG2, not cytotoxic) seem to display a similar therapeutic activity. Similarly, experimental models suggest that anti-PD-L1 antibodies have a better activity if they are cytotoxic but two anti-PD-L1 mAbs under clinical development, atezolizumab (an aglycosylated IgG1)
and durvalumab (a triple-mutated IgG1) are not cytotoxic and are both devoid of any effector function. A third, avelumab (a standard IgG1) has “normal” effector functions. For anti-PD-1 antibodies, experimental models suggest they have a better activity if they are not cytotoxic.\textsuperscript{76,77} Nivolumab and pembrolizumab, based on a γ4 isotype, are supposed not to be cytotoxic but their effector functions are not precisely known.\textsuperscript{78-80} A third anti-PD-1 mAb, pidilizumab, is an IgG1 with intact effector functions. Comparing the clinical efficacy of the different anti-PD-1 and anti-PD-L1 antibodies will be difficult because they could target different epitopes and are studied in very different clinical settings.

Combinations with immunomodulatory antibodies, Andrea van Elsas

The tumor microenvironment, endothelium, and other stromal elements exert potent immune suppression that cannot be overcome by PD-1 or CTLA-4 blockade alone. In addition, the mutational load or neoantigen potential of cancers represents an important factor determining the chances of durable immune rejection.\textsuperscript{81} To disrupt the tumor microenvironment and enhance neoantigen presentation, several widely used standard-of-care and experimental treatment modalities (e.g., anthracyclines, gemcitabine, oxaliplatin) can be used to improve the efficacy of immune checkpoint inhibitors. By promoting “immunogenic cell death” these provide a window of opportunity that extends the benefit of checkpoint inhibitors through enhancing T-cell activation and opening up immune evasive “cold” tumors to T-cell infiltration.\textsuperscript{82}

Ample evidence now indicates that durable clinical response to chemotherapy is dependent on tumor immunity, and the presence and quality of immune infiltrate is an independent indicator of successful response to chemotherapy. Durability of clinical response to targeted therapeutics is also likely enhanced by immune modulation.\textsuperscript{83} Radiotherapy is thought to induce immunogenic cell death and help generate an immune permissive tumor microenvironment, as confirmed by recent experimental work demonstrating the success of radioimmunotherapy.\textsuperscript{84,85}

Disclosure of potential conflicts of interest

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