DETAILS OF THE PROCEEDINGS

(1) ADJUVANTS

Monophosphoryl Lipid A (TLR4 Agonist)
Presenter: Mac Cheever, M.D.

Monophosphoryl lipid A (MPL or MPLA) is a component of lipopolysaccharide (LPS), or endotoxin, the first identified agonist to Toll-like receptor 4 (TLR4). LPS functions as a vaccine adjuvant but is considered too toxic for clinical use. However, purifying MPL from *Salmonella minnesota* endotoxin yields an excellent, low-toxicity adjuvant capable of activating macrophages and especially dendritic cells (DCs). It has been shown in animal models to elicit responses to antigens of low immunogenic potential such as malarial sporozoites. It has been administered by various routes and used in multiple formulations, including in combination with other adjuvants, and has been proposed for use as monotherapy to prevent viral, bacterial, and fungal disease. In this capacity, it may have a role in biodefense.

More than 120,000 doses have been administered to more than 50,000 human subjects. Already approved as a component of an HBV vaccine in the European Union, it is a safe adjuvant with a side-effect profile equivalent to that of alum. The "standard" HBV vaccine includes hepatitis B surface protein plus alum as adjuvant. Addition of MPL to the standard vaccine formulation stimulates a greater antibody response than alum alone. The standard HBV vaccine requires three doses to achieve protective responses in almost all patients. The addition of MPL provides protective antibody responses in almost all patients after two vaccinations. GlaxoSmithKline has presented similar data with a human papillomavirus (HPV) vaccine formulation with MPL as an adjuvant.

Dr. Cheever reported on two cancer vaccine trials that used MPL in combination with QS21. One involved the MAGE-A2 protein for melanoma and the other the HER2 protein in combination with QS21 and CpG against breast cancer.

MPL is available as a purified biologic consisting of several closely related molecules, although a pure synthetic TLR4 agonist, glucopranosyl lipid (GLA), is also available. The Infection Disease Research Institute in Seattle has expressed an interest in collaborating with investigators and a willingness to supply MPL at cost. The Institute's intention is to make it available for use as an adjuvant for vaccines in developing countries.

Dr. Cheever proposed using MPL as an adjuvant in combination with various antigens, noting that it is the "workhorse" of GlaxoSmithKline—the largest world-wide manufacturer of vaccines. MPL could be useful in the context of cancer vaccines.

Discussion

The other reviewers agreed that there has been a great deal of experience with this agent and that is was an effective and non-toxic adjuvant. MPL will probably not be approved as monotherapy, but vaccines that contain MPL such as HBV and HPV vaccines will be approved. There is such a

desperate need by academic researchers for cancer vaccines that once infectious disease vaccines containing MPL are approved, the infectious disease vaccines will be added to cancer vaccine regimens. Currently, GM-CSF is commonly used as a cancer vaccine adjuvant because it's available as a GMP agent, albeit for another purpose. It is highly likely that HBV and HPV vaccines containing MPL will likewise be used as components of academic cancer vaccines.

The synthetic version may be available from IDRI for research. It is not clear if it is currently being used in investigator-initiated trials or whether there is human data. One participant asked whether a drug master file for infectious diseases could be cross-referenced by cancer vaccine researchers. MPL is an older agent and is off patent.

Drew Pardoll, M.D., Ph.D., referred to a recent article in *Science* [Mata-Haro et al., The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science, 316(5831):1628-32, 2007] reporting that the low toxicity of MPLA, as compared to the parent compound LPS, is likely caused by the active suppression of proinflammatory activity.

Karolina Palucka, M.D., Ph.D., posited that MPL would be of strong interest to investigators studying DC vaccines.

Jeffrey Weber, M.D., Ph.D., said not much evidence is available that MPL alone stimulates T-cell activity. Not until CpG was added to the AS15 adjuvant combination were significant clinical and immunologic reactions seen.

Elizabeth Jaffee, M.D., referred to preclinical data indicating that TLR4 can affect DC activation.

Several participants brought up points related to TRIF and MyD88 signaling. TLR9 is very limited in the human and not expressed to a significant extent on conventional DCs. MPL is very interesting in the context of prophylactic cancer vaccines (e.g., MAGE and HER2).

Most participants agreed that MPL would most likely be part of a regimen consisting of multiple agents. Louis Weiner, M.D., emphasized the importance of having agents available that could be used to demonstrate important biologic consequences of manipulating signals in certain ways. MPL would be useful because of its restricted mechanism of action. Most agreed that lipopolysaccharide (LPS) is the best activator of DCs and would be interesting to include in a comparison or control arm. It is available from Dr. Anthony Suffredini's laboratory for research purposes.

It was mentioned that MPL really refers to two agents: the synthetic form and the natural form. Most information is available on the natural form. The purification procedure is reputed to be challenging.

References

- Gay NJ, Gangloff M. Structure and function of toll receptors and their ligands. Annu Rev Biochem, 76:141-165, 2007.

- Zanin-Zhorov A, Tal-Lapidot G, Cahalon L, Cohen-Sfady M, Pevsner-Fischer M, Lider O, Cohen IR. Cutting edge: T cells respond to lipopolysaccharide innately via TLR4 signaling. J Immunol, 179(1):41-44, 2007.
- Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TC. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science, 316:1628-1632, 2007.
- Elamanchili P, Lutsiak CM, Hamdy S, Diwan M, Samuel JJ. "Pathogen-mimicking" nanoparticles for vaccine delivery to dendritic cells. Immunother, 4:378-395, 2007.
- Nevens F, Zuckerman JN, Burroughs AK, Jung MC, Bayas JM, Kallinowski B, Rivas EF, Duvoux C, Neuhaus P, Saliba F, Buti M, Zarski JP, Pons F, Vanlemmens C, Hamtiaux V, Stoffel M. Immunogenicity and safety of an experimental adjuvanted hepatitis B candidate vaccine in liver transplant patients. Liver Transpl, 10:1489-1495, 2006.
- Giannini SL, Hanon E, Moris P, Van Mechelen M, Morel S, Dessy F, Fourneau MA, Colau B, Suzich J, Losonsky G, Martin MT, Dubin G, Wettendorff MA. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminum salt combination (AS04) compared to aluminium salt only. Vaccine, 24(33-34):5937-5949, 2006.
- Baldridge JR, McGowan P, Evans JT, Cluff C, Mossman S, Johnson D, Persing D. Taking a Toll on human disease: Toll-like receptor 4 agonists as vaccine adjuvants and monotherapeutic agents. Review. Expert Opin Biol Ther, 4:1129-1138, 2004.
- Atanackovic D, Altorki NK, Stockert E, Williamson B, Jungbluth AA, Ritter E, Santiago D, Ferrara CA, Matsuo M, Selvakumar A, Dupont B, Chen YT, Hoffman EW, Ritter G, Old LJ, Gnjatic S. Vaccine-induced CD4+ T cell responses to MAGE-3 protein in lung cancer patients. J Immunol, 172(5):3289-3296, 2004.
- Ismaili J, Rennesson J, Aksoy E, Vekemans J, Vincart B, Amraoui Z, Van Laethem F, Goldman M, and Dubois PM. Monophosphoryl lipid A activates both human dendritic cells and T cells. J Immunol, 168:926-932, 2002.
- Sondak VK, Liu PY, Tuthill RJ, Kempf RA, Unger JM, Sosman JA, Thompson JA, Weiss GR, Redman BG, Jakowatz JG, Noyes RD, Flaherty LE. Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: overall results of a randomized trial of the Southwest Oncology Group. J Clin Oncol, 20(8):2058-2066, 2002.
- Baldrick P, Richardson D, Elliott G, Wheeler AW. Safety evaluation of monophosphoryl lipid A (MPL): an immunostimulatory adjuvant. Regul Toxicol Pharmacol, 35:398-413, 2002.
- Childers NK, Miller KL, Tong G, Llarena JC, Greenway T, Ulrich JT, Michalek SM. Adjuvant activity of monophosphoryl lipid A for nasal and oral immunization with soluble or liposome-associated antigen. Infect Immun, 68(10):5509-5516, 2000.
- Chase, JJ, Kubey W, Dulek MH, Holmes CJ, Salit MG, Pearson FC, 3rd, Ribi E. Effect of monophosphoryl lipid A on host resistance to bacterial infection. Infect Immun, 53:711-712, 1986.

CpG (TLR9 Agonist)
Presenter: Ellis Reinherz, M.D.

CpG belongs to a category of drugs called immunomodulators. The nature of the agents is well defined in the literature. GMP-grade synthesis and purification are simple and economical. The distribution of the receptor is quite distinct. In humans, it is expressed on B cells and plasmacytoid dendritic cells (DCs). In the mouse, it is expressed on B cells, monocytes, and all DCs. These species-based differences make it a bit difficult when discussing preclinical data.

The biology is straightforward. The pathway activates through MyD88. Interaction of the agent with the target, toll-like receptor 9 (TLR9), leads to B-cell proliferation and differentiation, maturation of plasmacytoid DCs, and activation of natural killer (NK) cells. Proinflammatory cytokine release and Treg generation are problematic, however, because they counteract many of the desirable effects.

In preclinical studies, TLR9 agonist as monotherapy seems to work best when injected into or around small tumors. It has been used in various combination therapies, all of which showed a greater effect than CpG-ODN (oligodeoxynucleotides) given alone.

Toxicology studies in rats showed the presence of mononuclear cell infiltrates in liver, kidney, spleen, and bone marrow. Cytokine storms and proinflammatory cytokine increases in serum were seen at higher doses. Autoimmunity has not been reported, but CpG reportedly increases autoimmunity observed in lupus, multiple sclerosis, colitis, and arthritis mouse models.

The agent has been studied in phase I and II trials as monotherapy, in combinations, and as a vaccine adjuvant. Results vary, depending on the CpG studied. (“Not all CpGs are created equal.”)

In humans, CpG has demonstrated activity with few adverse events (AEs). Most reported AEs were tolerable local effects at the injection site. Several phase 3 trials are getting under way:

1. Randomized trial of gemcitabine/cisplatin + PF-3512676 vs. gemcitabine/cisplatin alone in patients with advanced non–small-cell lung cancer (NSCLC) (Pfizer/Coley).

2. Randomized trial of paclitaxel/carboplatin + PF-3512676 vs. paclitaxel/carboplatin alone in patients with advanced NSCLC (Pfizer/Coley).

3. Adjuvant therapy with recombinant MAGE-A3 protein + CPG7909 in MAGE-A3–positive patients with early stage, completely resected stage IB, II, or IIIA NSCLC (GlaxoSmithKline/Coley).

However, with regard to 1 and 2 above, both trials have been discontinued for NSCLC, as reported by Jesus Gomez-Navarro at this meeting. More specifically, the scheduled interim analysis of the phase 3 clinical trials by an independent Data Safety Monitoring Committee (DSMC) found no evidence that PF-3512676 produced additional clinical efficacy over that

achieved with the standard cytotoxic chemotherapy regimen alone. The DSMC concluded that the risk-benefit profile did not justify continuation of the trials.

According to Dr. Reinherz, this agent seems to be readily producible in a synthetic form. It is largely tolerable with minor side effects. An important limitation is its activation of Tregs, a phenomenon that counteracts some desired effects. It might be possible to combine CpG with other agents to counteract this.

The other reviewers pointed out that CpG has not been evaluated in breast or prostate cancer trials. They agreed that if this agent is to move forward, it would have to be used with agents that inhibit Tregs. Despite the research activity involving CpG, it is not generally available. Dr. Weiner suggested that CpG might not meet milestones used for most oncology agents. He suggested thinking about ways to incorporate such activators in vaccine studies.

Dr. Weber recalled that several small phase 2 studies have involved CpG. He mentioned Prof. Pedro Romero's study comparing peptide/IFA, and CpG as adjuvants. T-cell and tetramer responses were boosted with CpG. Near the mean toxic dose (MTD), no antitumor activity was observed when given intravenously. As monotherapy, it does not appear very promising although it may be useful in combination treatments.

Jay Berzofsky, M.D., Ph.D., mentioned that suppressor-type CpGs could inhibit Tregs. Any type of immunization induces some counterbalancing Treg activity. It is not clear whether CpG induces Tregs more than other vaccines do.

One participant observed that TLRs are also present on tumor cells. What is the effect of these agonists on tumor cells? Are there data showing that solid tumors express TLR9? Theresa Whiteside, Ph.D., referred to her own data involving squamous cell carcinoma.

Dr. Palucka emphasized that such products could have tremendous value as adjuvants. This CpG has been studied extensively. Nora Disis, M.D., said that local injection of CpGs is relatively unexplored and might be more efficacious than systemic delivery. She mentioned that one group observed interesting results with intranodal injection for lymphoma.

Several participants mentioned the importance of testing immunotherapies based on biologically relevant end points. Trying to reach end points in very ill patients is probably not going to show promising results. CpG is backed with sound science, but attempts to develop it with commercial intent led to the agent's becoming unavailable to those working on proof of concept. Many people remain interested in learning how such agents work. Having it available for studies that capitalize on its biologic strengths would be very useful.

Others recommended focusing on local rather than systemic administration of CpG and similar agents.

Crystal Mackall, M.D., asked how to select the most promising of the three CpG classes. All agreed that this is an important question. It was suggested that Dr. Klinman of the National

Cancer Institute could advise on this point. Jay Berzofsky, M.D., Ph.D., observed that Dr. Klinman uses a different nomenclature.

After completing discussion of each agent, the participants discussed the relative ranking of agents discussed to that point in the workshop and gave a relative rank by general consensus and acclamation. The general consensus was that CpG should rank higher than MPL in the priority list of adjuvants.

References

- Krieg, A. M. Development of TLR9 agonists for cancer therapy. J Clin Invest, 117:1184-1194, 2007.
- Kanzler, H., Barrat, F. J., Hessel, E. M., and Coffman, R. L. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. Nat Med, 13:552-559, 2007.
- Molenkamp, B. G., van Leeuwen, P. A., Meijer, S., Sluijter, B. J., Wijnands, P. G., Baars, A., van den Eertwegh, A. J., Scheper, R. J., and de Gruijl, T. D. Intradermal CpG-B activates both plasmacytoid and myeloid dendritic cells in the sentinel lymph node of melanoma patients. Clin Cancer Res, 13:2961-2969, 2007.
- Valmori, D., Souleimanian, N. E., Tosello, V., Bhardwaj, N., Adams, S., O'Neill, D., Pavlick, A., Escalon, J. B., Cruz, C. M., Angiulli, A., Angiulli, F., Mears, G., Vogel, S. M., Pan, L., Jungbluth, A. A., Hoffmann, E. W., Venhaus, R., Ritter, G., Old, L. J., and Ayyoub, M. Vaccination with NY-ESO-1 protein and CpG in Montanide induces integrated antibody/Th1 responses and CD8 T cells through cross-priming. Proc Natl Acad Sci U S A, 104:8947-8952, 2007.
- Krieg, A. M. Therapeutic potential of Toll-like receptor 9 activation. Nat Rev Drug Discov, 5:471-484, 2006.
- CpG 7909: PF 3512676, PF-3512676. Drugs R D, 7:312-316, 2006.
- Pashenkov, M., Goess, G., Wagner, C., Hormann, M., Jandl, T., Moser, A., Britten, C. M., Smolle, J., Koller, S., Mauch, C., Tantcheva-Poor, I., Grabbe, S., Loquai, C., Esser, S., Franckson, T., Schneeberger, A., Haarmann, C., Krieg, A. M., Stingl, G., and Wagner, S. N. Phase II trial of a toll-like receptor 9-activating oligonucleotide in patients with metastatic melanoma. J Clin Oncol, 24:5716-5724, 2006.
- Link, B. K., Ballas, Z. K., Weisdorf, D., Wooldridge, J. E., Bossler, A. D., Shannon, M., Rasmussen, W. L., Krieg, A. M., and Weiner, G. J. Oligodeoxynucleotide CpG 7909 delivered as intravenous infusion demonstrates immunologic modulation in patients with previously treated non-Hodgkin lymphoma. J Immunother (1997), 29:558-568, 2006.
- Speiser, D. E., Lienard, D., Rufer, N., Rubio-Godoy, V., Rimoldi, D., Lejeune, F., Krieg, A. M., Cerottini, J. C., and Romero, P. Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. J Clin Invest, 115:739-746, 2005.
- Friedberg, J. W., Kim, H., McCauley, M., Hessel, E. M., Sims, P., Fisher, D. C., Nadler, L. M., Coffman, R. L., and Freedman, A. S. Combination immunotherapy with a CpG

oligonucleotide (1018 ISS) and rituximab in patients with non-Hodgkin lymphoma: increased interferon-alpha/beta-inducible gene expression, without significant toxicity. Blood, 105:489-495, 2005.

Resiquimod and 852A

Presenter: Louis M. Weiner, M.D.

The imidazoquinolinamines resiquimod and 852A are TLR7/8 agonists, which induce innate and adaptive immune responses. Their biology is similar to that of imiquimod (TLR7 agonist), which is currently FDA approved as a topical medication for basal cell skin cancer. Anecdotal reports have indicated that imiquimod is useful for managing some cases of melanoma with cutaneous metastases. Significantly, TLR7 distribution is similar to that of TLR9. Imiquimod also acts on TLR8 to a small extent, but not at achievable doses. Resiquimod induces production of interferon-alpha; Interleukins 6, 8, and 12; and TNF-alpha from DCs, monocytes, and macrophages. Activation stimulates the innate immune response and leads to subsequent Th1 cell-mediated immune responses.

Among the contemplated uses of resiquimod is as monotherapy for immune activation. This does not appear to be useful as a systemic approach because topical administration is required. It might also be used in combination with other chemotherapy agents or with antigen-specific antibodies. Another possibility would be use as a vaccine adjuvant. Based on information provided by 3M, resiquimod could be formulated for oral administration, although it is not clear that this would provide any advantage in a vaccine adjuvant setting.

A recent presentation at the American Society for Clinical Oncology meeting indicated that cytokine storm–type toxicities occur, but clinical responses have been observed in a variety of tumor types. This type of reaction could possibly be a harbinger of immunologic benefit, but more information would be required. Dr. Weiner opined that in an ideal world, either resiquimod or imiquimod would be developed as a means of exploring biologic activity, but how they compare with other agents is unknown at this point.

The Coley Pharmaceutical Group has taken over the TLR program from 3M. Modeling with CpGs is difficult because animals do not have the same TLR distribution.

Another TLR7 agonist is 852A, which stimulates plasmacytoid DCs and is administered as an intravenous solution. Scant data are available on 852A, although indications are that it may be more potent than resiquimod. Dudek et al. reported that clinical responses have been seen in carcinoid tumor, melanoma, and breast cancer.

Both resiquimod and 852A are relatively easy to manufacture and potentially available in various formulations.

In sum, Dr. Weiner said that having TLR7 agonists available would add to vaccine adjuvant options. Having topical and systemic formulations could also be useful. Resiquimod, however, might not be sufficiently distinct from imiquimod to warrant development unless a parenteral formulation is possible. Because of its potent immune activation and a demonstration of having

some activity in a phase I trial, 852A merits consideration for future clinical development. Such agents are being studied as a means of stimulating antigen-presenting cells and generating large numbers of T cells in the setting of adoptive T-cell therapy.

Discussion

George Prendergast, Ph.D., commented that TLR7 or TLR8 agonists are important components of current thinking; therefore, a role exists for CpG ligands and associated regulatory mechanisms. The imiquimods can also tamp down desirable responses.

The participants discussed the dearth of publications on some promising agents, for example, 852A. Much research goes unpublished. Several participants commented on the potential diversity of studies that could be done with these agents. The entire TLR program is in the hands of Coley Pharmaceutical Group, which has been cooperative about providing agents for small pilot trials and exchanging information. It might be possible to obtain additional information.

One participant asked whether any investigators have looked into injecting imiquimod into tumors, noting that this agent is approved for treating basal cell carcinoma topically and it induces major inflammatory responses. The notion of using these agents in a local fashion as opposed to systemically is very under-explored. Several people emphasized the importance of moving away from "drug" studies because they probably will not be useful for most immune therapies. Mixed TLR 7/8 agonists would be very interesting used locally. A robust series of studies is needed.

Dr. Pardoll cited the experience of Stengall, who used imiquimod topically (Aldara) over GVAX vaccination sites; the effects were dramatic. Type 1 interferons and other inflammatory cytokines increased, and biopsy of the vaccination site showed an inflammatory infiltrate. Additional data are being analyzed to learn whether Aldara enhanced the vaccine response.

It was suggested that the priority ranking should incorporate some flexibility so that as more is learned, priorities may be modified. Dr. Creekmore said it might be possible to obtain resiquimod/852A for the repository to make it more widely available through CTEP or DTP. The group was very interested in gaining access to this drug, although it was not clear that it would be ranked highly. All agreed that more information—unpublished data, in particular—is needed. Perhaps a confidentiality agreement could be executed to gain access to such data.

The participants ranked resiquimod/852A below CpG and MPL at this point.

References

- Mark KE, Corey L, Meng T-C, Magaret AS, Huang M-L, Selke S, Slade HB, Tyring SK, Warren T, Sacks SL, Leone P, Bergland VA, Wald A. Topical resiquimod 0.01% gel decreases herpes simplex virus type 2 genital shedding: a randomized, controlled trial. J. Inf. Dis, 195:1324–1331, 2007.
- Wu JJ, Huang DB, Tyring SK. Resiquimod: a new immune response modifier with potential as a vaccine adjuvant for Th1 immune responses. Antiviral Res, 64:79-83, 2004.

- Jones T. Resiquimod 3M. Curr Opin Investig Drugs, 4:214-218, 2003.
- Hengge UR, Benninghoff B, Ruzicka T, Goos M. Topical immunomodulators—progress towards treating inflammation, infection, and cancer. Lancet Infect Dis, 1:189-198, 2001.
- Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol, 7:179-190, 2007.
- Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol, 4:499-511, 2004.
- Dudek AZ, Yunis C, Kumar S, Harrison LI, Hawkinson RW, Miller JS. ASCO Annual Meeting Proceedings. J Clin Oncol, 23(16S, Part I of II June 1 Supplement):2515, 2005.

Flt3 Ligand

Presenter: Drew Pardoll, M.D., Ph.D.

Dr. Pardoll reported that much information is available on the Flt3 ligand, a hematopoietic growth factor that binds to the Flk2/Flt3 receptor tyrosine kinase in the c-kit/fms family. It demonstrates broad activity, but is notable for inducing the expansion and differentiation of all DC progenitors, especially interferon-producing killer and plasmacytoid DCs. Such discoveries have led to a slew of preclinical models in which it has been used systemically as a single agent, a vaccine adjuvant, or in conjunction with DC activators such as CpGs and anti-CD40. It is very clear that systemic administration of Flt3 ligand increases DC numbers in blood, secondary lymphoid tissues, and tumors. Some investigators have reported that it also increases DC numbers in the tumor but others have not been able to replicate this finding.

A great deal of preclinical and a small amount of clinical data are available. Scattered phase I/II reports have presented results of using Flt3 ligand alone, with peptide vaccines, as DC stimulators, and after bone marrow transplant. Giving the agent as an adjuvant with DC vaccines would be a basis for very interesting studies. Using Flt3 ligand with two peptides bumped up numbers of interferon-gamma–producing T cells.

Flt3 ligand appears to be reasonably well tolerated. Development of Sjögren's–type syndrome in one patient was reported in one study.

Immunex, which has merged with Amgen, terminated studies after trying several "drug-type" approaches to evaluating its efficacy as a single agent or with soluble CD40 ligand. Dr. Pardoll was not sure about the agent's current status. It appears that it has not been tested in a more biologically logical way, such as in conjunction with a DC activator and an antigen. Small studies in academic centers would be appropriate for some interesting immunologic studies such as local administration at the tumor site.

Discussion

Dr. Weber commented on the pattern of developing potential adjuvants as stand alone drugs and then terminating the studies when they do not show typical "drug" efficacy in a few clinical studies. Flt3 ligand is an interesting agent that merits more study based on its performance in early studies, but it is no longer available.

Another participant noted that developers of dendritic cell vaccines were interested in Flt3 ligand's capacity to mobilize DCs that could then be collected and manipulated *ex vivo.* Flt3 ligand would serve as a good base to which other agents could be added.

Frank Calzone, Ph.D., clarified that Amgen has made the agent available for preclinical studies. Clinical trials are a very expensive undertaking. The results of efficacy testing have not been encouraging to date.

Most participants agreed that if Flt3 ligand would be a very interesting agent to pursue, particularly in combination therapies.

One participant observed that when treating patients with proteins that have endogenous counterparts, one must consider immune responses to the proteins and resultant autoimmune response against important normal proteins. For an end-stage cancer patient, the risk might be acceptable.

Another person noted that Flt3 ligand is a very potent activator of thymic function and dramatically increases CD4+ T cells. This aspect of Flt3 ligand is underappreciated, but could be interesting for treating patients after bone marrow transplantation.

The group discussed the priority rankings of the adjuvants presented thus far. Flt 3 ligand is similar to CpG in the sense that it has profound and interesting activity, but clinical trials to date have used it in the wrong way and have not taken maximum account of its intrinsic biology. By voice acclamation, the agents were ranked thus: CpG, Flt3 ligand, MPL, resiquimod/852A. However, each agent was considered quite important.

References

- Matthews W, Jordan CT, Wiegand GW, Pardoll D, Lemischka IR. A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations. Cell, 65(7):1143-1152, 1991.
- Lynch DH, Andreasen A, Maraskovsky E, Whitmore J, Miller RE, Schuh JC. Flt3 ligand induces tumor regression and antitumor immune responses in vivo. Nat Med, 3(6):625-631, 1997.
- Marroquin CE, Westwood JA, Lapointe R, Mixon A, Wunderlich JR, Caron D, Rosenberg SA, Hwu P. Mobilization of dendritic cell precursors in patients with cancer by flt3 ligand allows the generation of higher yields of cultured dendritic cells. J Immunother, 25(3):278-288, 2002.

- Cui Y, Kelleher E, Straley E, Fuchs E, Gorski K, Levitsky H, Borrello I, Civin CI, Schoenberger SP, Cheng L, Pardoll DM, Whartenby KA. Immunotherapy of established tumors using bone marrow transplantation with antigen gene–modified hematopoietic stem cells. Nat Med, 9(7):952-958, 2003.
- Furumoto K, Soares L, Engleman EG, Merad M. Induction of potent antitumor immunity by in situ targeting of intratumoral DCs. J Clin Invest, 113(5):774-783, 2004.
- Chen W, Chan AS, Dawson AJ, Liang X, Blazar BR, Miller JS. FLT3 ligand administration after hematopoietic cell transplantation increases circulating dendritic cell precursors that can be activated by CpG oligodeoxynucleotides to enhance T cell and natural killer cell function. Biol Blood Marrow Transplant, 11(1):23-34, 2005.
- Davis ID, Chen Q, Morris L, Quirk J, Stanley M, Tavarnesi ML, Parente P, Cavicchiolo T, Hopkins W, Jackson H, Dimopoulos N, Tai TY, MacGregor D, Browning J, Svobodova S, Caron D, Maraskovsky E, Old LJ, Chen W, Cebon J. Blood dendritic cells generated with Flt3 ligand and CD40 ligand prime CD8+ T cells efficiently in cancer patients. J Immunother, 29(5):499-511, 2006.

Poly I:C and Poly-ICLC

Presenter: Anna Karolina Palucka, M.D., Ph.D.

Dr. Palucka explained that poly I:C is double-stranded polyinosinic:polycytidylic acid. When stabilized with poly-L-lysine and carboxymethylcellulose, it is known as poly-ICLC, which is more stable and, in that regard has greater activity. The target for the agents is TLR-3. *In vivo* preclinical studies have demonstrated that they activate human DCs, improve antigen presentation, and enhance Th1 polarization. In animal models, they exert an adjuvant effect when administered with cancer or infectious disease vaccines. They also improve cross-priming and activate natural killer cells. In humans, they are strong activators of Th1 responses, CD8 T cells, and natural killer cells.

Dr. Palucka highlighted clinical experience, stating that monotherapy has not been very effective. Recently, Ampligen (polyI:polyC12U) was tested for activity against viral infections, including HIV, SARS, HPV, and HCV, because of its demonstrated antiviral activity and its ability to stimulate production of type 1 interferon and activate RNase-L (antiviral). Clinicaltrials.gov lists trials accruing HIV and chronic fatigue syndrome patients for study.

Ongoing phase I/II trials of Hiltonol (poly-ICLC) involve patients with malignant gliomas. The agent is also being tested in prostate cancer patients for adjuvant effect with a MUC1 100-mer peptide vaccine.

In all likelihood, poly I:C and poly-ICLC would be of limited utility as systemic agents for monotherapy, but they might be useful adjuvants for cancer vaccines based on *ex vivo* DCs or *in vivo* as an adjuvant, although this remains to be seen. More work should also be done to investigate the efficacy of immunotherapy administered within or around the tumor site.

According to Dr. Palucka, both agents might be available for use in clinical trials. She cautioned that TLR4 and TLR3 agonists are not always beneficial in humans; therefore, a great deal of thought needs to go into understanding the rationale for combining different biologics, as well as dosing and kinetics.

Discussion

Theresa Whiteside, Ph.D., raised a point about the interaction between DCs and up-regulation of Tregs.

Dr. Ho reiterated that these agents have been around for some time. Newer versions are more stable. Some trials are studying their use in chronic fatigue syndrome.

Dr. Weber noted that using CD40 agonist with poly I:C gives good clinical effect and immunologic responses. According to Dr. Cheever, poly I:C was discovered and used clinically before TLRs were defined at the molecular level.

Dr. Berzofsky pointed out that poly I:C and poly-ICLC are among the few TLR ligands that work exclusively on one receptor type (i.e., TLR3 that acts through TRIF rather than MyD88 as the other TLRs do). Therefore, it does not duplicate the other TLR ligands on the list of agents under consideration; it would be complementary.

The participants discussed the ranking of adjuvants considered thus far. Dr. Cheever suggested that if the company is making an agent broadly available, it should be lower on the priority list. Even if the agent is exceedingly valuable for study it does not need the attention of this group. Dr. Palucka opined that, from the standpoint of vaccine efficacy and clinical utility, she would place it above CpG in the rankings, but because it seems to be more broadly available, it probably does not merit that position on the priority list.

By voice acclamation, the agents were ranked thus: CpG, Flt3 ligand, poly I:C or poly-ICLC, MPL, resiquimod/852A.

References

- Adams M, Navabi H, Jasani B, Man S, Fiander A, Evans AS, Donninger C, Mason M. Dendritic cell (DC) based therapy for cervical cancer: use of DC pulsed with tumour lysate and matured with a novel synthetic clinically non-toxic double stranded RNA analogue poly [I]:poly [C(12)U] (Ampligen R). Vaccine, 30;21(7-8):787-790, 2003.
- Choe J, Kelker MS, Wilson IA. Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. Science, 22;309(5734):581-585, 2005.
- Ewel CH, Urba WJ, Kopp WC, Smith JW, Steis RG, Rossio JL, Longo DL, Jones MJ, Alvord WG, Pinsky CM, et al. Polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose in combination with interleukin 2 in patients with cancer: clinical and immunological effects. Cancer Res, 52(11):3005-3010, 1992.

- Giantonio BJ, Hochster H, Blum R, Wiernik PH, Hudes GR, Kirkwood J, Trump D, Oken MM. Toxicity and response evaluation of the interferon inducer poly ICLC administered at low dose in advanced renal carcinoma and relapsed or refractory lymphoma: a report of two clinical trials of the Eastern Cooperative Oncology Group. Invest New Drugs, 19(1):89-92, 2001.
- Gitlin L, Barchet W, Gilfillan S, Cella M, Beutler B, Flavell RA, Diamond MS, Colonna M. Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. Proc Natl Acad Sci U S A, 30;103(22):8459-8464, 2006.
- Kawai T, Akira S. Innate immune recognition of viral infection. Nat Immunol, 7(2):131-137, 2006.
- Mailliard RB, Wankowicz-Kalinska A, Cai Q, Wesa A, Hilkens CM, Kapsenberg ML, Kirkwood JM, Storkus WJ, Kalinski P. Alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. Cancer Res, 64(17):5934-5937, 2004.
- Uematsu S, Akira S. Toll-like receptors and Type I interferons. J Biol Chem, 282(21):15319-15323, 2007.
- Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol, 5(7):730-737, 2004.

Interleukin-12 (IL-12)
Presenter: Jeffrey Weber, M.D., Ph.D.

Interleukin-12 is a cytokine that binds to IL-12 receptor on natural killer cells, T cells, DCs, and macrophages. It promotes interferon-gamma release and induces Th1 polarization and proliferation of interferon-gamma–expressing T cells. It has anti-angiogenic activity and, according to recent reports, a role in autoimmunity, although it is likely that IL-23 is the more important factor.

IL-12 plays a central role in resistance to mycobacterial and intracellular pathogens (e.g., parasites). It also plays an important part in anticancer development and immunity in animal systems. Nevertheless, it has not demonstrated sufficient clinical activity as a stand-alone drug to warrant further development according to the standard oncology paradigm. It was originally developed as a systemic cytokine, but it proved challenging to administer safely.

This agent is an exceedingly potent immune adjuvant. It can be incorporated into vaccines or added at the local site. A handful of phase I and II studies have suggested that IL-12 used alone has modest efficacy in melanoma and renal cell carcinoma. Benefit might have been associated with elevated interferon-gamma levels. Reported adverse events included hepatitis, fevers, and cytokine storm. One septic death occurred. Several trials were halted prematurely because no supply of IL-12 was available, although the investigators very much wanted to continue the work because of interesting results.

Based on murine and human data, IL-12 appears to have excellent potential as either adjunctive cytokine therapy or as an adjuvant in a vaccine approach. It could be delivered locally via viral or other plasmid vectors. Its use as an adjuvant could both polarize Th1 responses and augment CD8 responses in any antigen-specific strategy. No phase III data are available.

Discussion

One meeting participant said, "It is among the most interesting vaccine adjuvants I've ever tested." Dr. Weiner concurred, stating that the whole research community has wanted access to this protein for a long time.

Dr. Weber said that giving IL-12 at the vaccination site can cause systemic effects. Dr. Pardoll noted concerns about whether the half-life of IL-12 is sufficiently long to garner an effect when administered locally. Dr. Weber responded that admixing IL-12 with alum prolongs the half-life and augments clinical response in murine models.

Dr. Creekmore said that CTEP has a small amount of IL-12.

Steve Hermann, Ph.D., pointed out that all the agents discussed thus far are toxic if administered intravenously and quite toxic if administered subcutaneously. Nora Disis, M.D., reported on a study using IL-12 delivered intraperitoneally. Another participant asked if any trials have been planned for local delivery in bladder cancer. Because the drug is no longer available, no trials are planned.

Dr. Hermann said that Wyeth plans to donate its remaining vials of IL-12 to the National Cancer Institute (NCI). Dr. Creekmore confirmed that NCI has received 4,000 vials and is expecting more, plus a supply of placebo. He reported on the status of processing and recertification of this supply of IL-12. He cautioned that after distributing the agent to finish the prematurely terminated studies, the amount left will not be large. A manufacturing agreement might be in the works.

The participants discussed toxicities associated with systemic administration of IL-12, including a recent report of central nervous system effects when given in low doses to patients with Kaposi's syndrome. Toxicities are dependent on dose and route of administration. Among the topics covered were possible paths forward based on local administration, vector delivery with adenovirus or avipox, or combining it with other agents, including IL-2. One participant cautioned that vector work is quite risky. Giving IL-12 as a cancer vaccine adjuvant would allow use of IL-12 concentrations that would not be highly toxic.

Kimberly Benton, Ph.D., said that IL-12 is a complicated molecule that has not been studied in the right way. She exhorted the group to consider strategies to learn more about it.

Another participant mentioned Seeger's work in neuroblastoma and ways to achieve prolonged release with local injection. One person spoke about slow release of IL-12 via microspheres in a mouse model.

Dr. Weiner summed up, saying this agent has generated enormous enthusiasm in the investigator community. Industry has had trouble understanding its value because the developmental path is not clear. Dr. Creekmore estimated that some 9,000 or 10,000 vials will be available, but the supply will probably run out in a few years. As was previously done with IL-7, the NCI might be able to manufacture a pilot lot of IL-12, although this would be very expensive. The best approach, he suggested, might be to work with the company for manufacture. Dr. Weiner agreed that a significant, pent-up demand exists for this agent; the existing supply will likely be depleted in short order. Dr. Jamie Zwiebel of CTEP said that once the quantity of IL-12 available is known, it might be possible to solicit studies and then prioritize them.

Dr. Weiner said that a small firm is interested in producing GMP-grade IL-12 but would like some idea of how much demand would exist.

Dr. Walter Urba requested more information about the studies that will be receiving IL-12. It would be important to confirm that these studies are properly designed to capitalize on the strengths of immunotherapeutic agents. For example, it would not be appropriate to study the agent in patients with advanced disease.

By voice acclamation, the priority ranking of adjuvants was determined to be IL-12, CpG, Flt3 ligand, poly I:C or poly-ICLC, MPL, resiquimod/852A.

References

- Fallarino, F., Uyttenhove, C., Boon, T. & Gajewski, T. F. Endogenous IL-12 is necessary for rejection of P815 tumor variants *in vivo*. J. Immunol, 156:1095-1100, 1996.
- Smyth, M. J., et al. Differential tumor surveillance by natural killer (NK) and NKT cells. J. Exp. Med, 191:661-668, 2000.
- Brunda, M. J., et al. Antitumor and antimetastatic activity of interleukin-12 against murine tumors. J. Exp. Med, 178:1223-1230, 1993.
- Noguchi, Y., Jungbluth, A., Richards, E. C. & Old, L. J. Effect of interleukin-12 on tumor induction by 3-methylcholanthrene. Proc Natl Acad Sci U S A, 93:11798-11801, 1996.
- Nanni, P., et al. Combined allogeneic tumor-cell vaccination and systemic interleukin-12 prevents mammary carcinogenesis in HER-2/neu transgenic mice. J Exp Med, 194: 1195-1205, 2001.
- Colombo, M P, Trinchieri G. Interleukin-12 in anti-tumor immunity and immunotherapy. Cytokine Growth Factor Rev, 13:155-168, 2002.
- Voest EE, et al. Inhibition of angiogenesis *in vivo* by interleukin-12. J Natl Cancer Inst, 87:581-586, 1995.
- Cebon J, Jager E, Shackleton MJ, Gibbs P, Davis ID, Hopkins W, Gibbs S, Chen Q, Karbach J, Jackson H, MacGregor DP, Sturrock S, Vaughan H, Maraskovsky E, Neumann A, Hoffman E, Sherman ML, Knuth A. Two phase I studies of low dose recombinant human IL-12 with Melan-A and influenza peptides in subjects with advanced malignant melanoma. Cancer Immun, 3:7, 2003.

- Peterson AC, Harlin H, Gajewski TF. Immunization with Melan-A peptide-pulsed peripheral blood mononuclear cells plus recombinant human interleukin-12 induces clinical activity and T cell responses in advanced melanoma. J Clin Oncol, 21(12):2342-2348, 2003.
- Hamid O, Solomon JC, Scotland R, Garcia M, Sian S, Ye W, Groshen SL, Weber JS. Alum with interleukin-12 augments immunity to a melanoma peptide vaccine: correlation with time to relapse in patients with resected high-risk disease. Clin Cancer Res, 13(1):215-222, 2007.
- Gollob JA, Mier JW, Veenstra K, McDermott DF, Clancy D, Clancy M, Atkins MB. Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-gamma induction is associated with clinical response. Clin Cancer Res, 6(5):1678-1692, 2000.

Interleukin-4 (IL-4)

Presenter: Theresa Whiteside, Ph.D., ABMLI

Interleukin-4 (IL-4) structurally resembles GM-CSF (granulocyte-macrophage colony-stimulating factor) and has 20% homology with IL-13. It targets a broad variety of cells that express IL-4 receptor, including B cells, T cells, natural killer cells, monocytes, and various tissue cells. It exerts a broad range of biologic effects, including allergic-type inflammation, especially of the eye, by causing mast cells to release histamine.

This cytokine signals through the IL-4 receptor, of which there are two types. The classical type I receptor, expressed on hematopoietic cells, consists of an IL-4 receptor alpha chain and a gamma chain. Type II receptor, expressed on cancer cells, consists of the IL-4 receptor alpha chain plus an IL-13 receptor alpha chain; therefore type II IL-4 receptor also binds IL-13.

In vitro studies have demonstrated that IL-4 suppresses growth of some IL-4 receptor–expressing tumor cells but promotes growth in others (e.g., head and neck squamous cell carcinoma). Dr. Whiteside summarized the cumulative preclinical experience with the agent, which is an important cytokine for differentiation and maturation of T cells and DCs.

The toxicity profile is well defined. The maximum tolerated dose (MTD) has been defined. When given in small doses, it appears to be safe and well tolerated. Only phase I and II clinical studies have been done. It has been given as monotherapy to more than 300 patients with advanced malignancies and showed no antitumor clinical efficacy. When given in combination with GM-CSF to patients with metastatic disease, however, it demonstrated some efficacy: one partial response, eight stable disease (8.5 mo), and 12 progressive disease. Hepatotoxicity has been reported rarely. It has also been used in vectored studies, yielding immunologic responses in some patients; one glioma patient had a transient response and survived for 10 months.

IL4 conjugated to diphtheria or *Pseudomonas* toxin has also been studied. Such fusion proteins are highly toxic to tumor cells. No objective clinical responses were observed per the literature.

This cytokine appears to have some other interesting effects. For example, in murine models, it can protect T cells from suppression by Tregs, presumably by up-regulating BCL2. When used

in autoimmune diseases such as systemic lupus, it exhibits paradoxical effects by promoting Th2 responses (autoantibody) while exerting a T cell–suppressive effect.

Dr. Whiteside speculated that IL-4 could potentially be used as an adjuvant for cancer vaccines, perhaps in combination with other cytokines, to increase the number and activity of antigen-presenting cells. In hematopoietic cell transplant, it could be used to ameliorate graft-versus-host disease and to augment antitumor Th1/Th2 responses. Another potential use would be in chronic inflammatory conditions, for modulating Th1/Th2 balance, as a way to explore the agent's anti-inflammatory activities. It is critical for many research groups in *ex vivo* culture regimens of myeloid DCs or IL-4 polarized CD4+ T cells.

Discussion

Dr. Ho reported that the most likely application of this cytokine would be for local delivery or *in vitro* use. He noted that it is available. Dr. Palucka reported that although several investigators are moving away from using IL-4 to generate DCs, in favor of interferon, many studies are still ongoing. Nevertheless, because clinical grade IL-4 is available, it should have lower priority than other agents discussed during the meeting.

Most agreed that its potential for *in vivo* use as a cancer adjuvant was limited. It is primarily useful as a T-cell growth factor. IL-4 has been around almost 20 years, but researchers do not really understand its effects on different subsets of cells. Dr. Berzofsky mentioned its usefulness for studying autoimmunity and skewing the immune response away from Th1.

By voice acclamation, the view was that IL-4 is interesting and potentially quite valuable, but consensus was to place IL-4 at the bottom of the list of adjuvants in priority.

References

- Leach MW, Rybak, ME, Rosenblum IY. Safety Evaluation of Recombinant Human Interleukin-4 II. Clinical Studies. Clin Immunol Immunopath, 83:12-14, 1997.
- Whitehead RP, Lew D, Flanigan RC, Weiss GR, Roy V, Glode ML, Dakhil SR, Crawford ED. Phase II trial of recombinant human interleukin-4 in patients with advanced renal cell carcinoma: a Southwest Oncology Group Study. J Immunother, 25(4):352-358, 2002.
- Gitlitz, BJ, Figlin RA, Kiertscher SM, Moldawer N, Rosen F, Roth MD. Phase I trial of granulocyte macrophage-colony stimulating factor and interleukin-4 as a combined immunotherapy for patients with cancer. J Immunother, 26(2):171-178, 2003.
- Okada H, Lieberman FS, Edington HD, Witham TF, Wargo MJ, Cai Q, Elder EH, Whiteside TL, Schold Jr CS, Pollack IF. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of recurrent glioblastoma: preliminary observations in a patient with a favorable response to therapy. J Neurooncol, 64:13-20, 2003.
- Garland L, Gitlitz B, Ebbinghaus S, Pan H, de Haan H, Puri RK, Von Hoff D, Figlin R. Phase I trial of intravenous IL-4 *Pseudomonas* exotoxin protein (nbi-3001) in patients with advanced solid tumors that express the il-4 receptor. J Immunother, 28(4):376-381, 2005.

- Fowler DH, Odom J, Steinberg SM, and 17 others. Phase I clinical trial of costimulated, IL-4 polarized donor CD4+ T cells as augmentation of allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant, 12:1150-1160, 2006.
- Pace L, Rizzo S, Palombi C, Brombacher F, Doria G. Cutting edge: IL-4-induced protection of CD4+CD25- Th cells from CD4+CD25+ regulatory T cell-mediated suppression. J Immunol, 176:3900-3904, 2005.

Discussion of Adjuvant Prioritization

By voice acclamation, the priority ranking of all the adjuvants discussed was determined to be:

1. IL-12.
2. CpG Flt3 ligand.
3. poly I:C and/or poly-ICLC.
4. MPL.
5. resiquimod/852A.
6. IL-4.

Dr. Pardoll expressed some concern about relying on an "Iowa Caucus" approach because even the agents at the bottom of the list are very interesting and have potential application in particular settings.

Any agents that merit discussion at this meeting are of potentially great value. The final priority ranking should be a means of reflecting both value and availability. Because the priorities are based on incomplete knowledge, the process should be a dynamic, ongoing one that can be revised as more data appear. The prioritization is not intended to reflect the overall potential of these agents; rather, the priorities should be deemed a recommendation to NCI about agents that should be made available for wider study. For example, if a very exciting agent is broadly available, it should receive a lower priority rank. It was agreed that cost should not be a factor when assessing availability. Purchasing an agent, even at great cost, is likely to be less expensive than manufacturing it. As a possible outcome of this meeting, NCI might be convinced to produce or obtain an agent, or industry might be stimulated to reinvigorate or refocus its efforts.

The group questioned the ranking of poly I:C. The ranking reflected a perception that the agent is potentially broadly available. Several suggested that poly I:C should be ranked below MPL, which is not commercially available. MPL seems to be the workhorse of GSK's vaccines going forward. It is nontoxic and can be combined with virtually every other adjuvant. "Academics should have access to it like water," stated one participant.

Dr. Pardoll emphasized the importance of establishing an ongoing process to priority setting. Dr. Cheever expressed a hope that the group could be involved in subsequent workshops, but no commitment has been made for additional meetings. The prioritization focus should be on drugs needed in the clinic now rather than on a common desire to conduct further preclinical work. The participants briefly discussed phase 0 studies.

Despite its interesting biology, 852A has not made it to the clinic because the commercial entity no longer wants to develop it.

Sufficient quantities of IL-4 are available to sustain existing programs. There was consensus that IL-4 is of lower priority than the other adjuvants.

IL-12 is also an antiangiogenic compound. As such, it could follow a different development pathway.

Dr. Raj Puri said that the FDA sees many trials that use IL-4 and other cytokines to activate DCs.

Dr. Berzofsky said that for DC generation, IL-15 and certain interferons might be better than IL-4. However, until IL-15 becomes available, IL-4 is the gold standard and will be needed for a long time to come.

Dr. Weiner said that MPL is a potentially useful adjuvant that would be of broad interest. More people would want access to MPL than to poly I:C for their vaccine studies. He recommended a higher priority for MPL. Other participants agreed that MPL is a useful agent but it does not have the intellectual interest of some other agents.

Dr. Urba suggested, since it is considered to be more broadly available, that poly I:C should appear below resiquimod on the list.

Several participants recommended creating a scientific list informed by scientific priorities. It must reflect the needs of general immunotherapy community as well as limitations of availability. Ultimately the priority rankings for adjuvants were not changed at the workshop.