***Q&A for ACT Educational Webinar on “Regulatory and Scientific Considerations for the Nonclinical Safety Assessment of Prophylactic and Therapeutic Vaccines”, presented by Jayanthi Wolf, David Clarke and Karissa Adkins on September 20th, 2017.***

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| **Question** | **Answer** |
| I have typically used rabbits for conjugates and subunit vaccines, but have rumors of some issues with idiopathic responses in rabbits lately, but have not seen anything in the literature about this. Thoughts? | We have not experienced idiopathic responses in rabbits. |
| Follow up on the rabbit idiopathic response question - I have very little information other than some colleagues insisting rabbits do not tolerate conjugate vaccines well/have rare tox w/ these products not correlated clinically. I've never seen this. | Thank you for your comment. |
| In prophylactic vaccine, is the clinical booster dose considered in the N+1 rule? | The clinical booster dose (assuming that the booster is included in the initial regimen) is included in the N+1 rule. For booster doses that might be needed several years after the initial regimen, the booster dose is not typically included in the N+1 rule. |
| Can you add some more detail on when integration studies would be required for a plasmid based therapeutic vaccine? | Based on published studies analyzing the frequency with which DNA plasmids persist and integrate, integration studies are warranted only when plasmid persists in any tissue of any animal at levels exceeding 30,000 copies per microgram of host DNA by study termination. A typical integration study will assess all tissues containing persisting DNA plasmid. Unintegrated plasmid DNA may be separated from high molecular weight genomic DNA by gel purification. Concatamer may be eliminated by restriction endonuclease digestion targeting a rare motif present in the DNA plasmid. Specifically designed PCR primers may be used to confirm integration and identify genomic integration sites.  References:  Ledwith et al., Intervirology. 2000;43(4-6):258-72.  Wang Z, Gene Ther. 2004 Apr;11(8):711-21.  US FDA’s Guidance for Industry: Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events (2006) |
| Adjuvant alone control group. Could it be avoided if adjuvant is well known? (e.g. alum | Yes, for adjuvants that are well-studied from a toxicology perspective and the toxicology data can be referenced in regulatory submissions, an adjuvant-alone group does not need to be included in the toxicology study. |
| If indicated for pregnant woman, could 2 species be requested by HAs due to different approached by US and EU health authorities? | There is no requirement to use 2 species for DART studies with vaccines. However, it is possible that for some specific vaccines to be indicated for maternal use, regulatory authorities might request use of two species (e.g. rats, rabbits) for DART studies based on potential safety concerns that cannot be addressed in a single species. This topic should be discussed with regulatory authorities during development (Type B/C meetings with FDA and Scientific Advice with EMA). |
| Is dosing in lactation a must have? non-rodents (e.g. rabbits) very delicate animal when littering....  Is dose adm during lactation phase an expectation for DART studies with vaccines? | Dosing during lactation is not mandatory and not typically done in vaccine DART studies. The vaccination regimen must optimize maternal antibody titers throughout the embryonic, fetal, and early post-natal periods. Antibodies transferred during gestation to the infant remain in the early post-natal period. |
| Is there a recommendation on the timing for immunization during lactation phase? | Administering during the early phase of lactation could be considered (e.g. Lactation Day 7 for rodents).  Reference: HPV Vaccine: L.D. Wise et a. Birth Defects Res B Dev Reprod Toxicol. 2008 Dec;83(6):561-72. |
| Repeat Dose Toxicity study in rodents, is it 20/group or 20/sex/group? | For rodents, it’s recommended to use 10/gender/group per necropsy. Because there are usually two necropsies (end of dosing and recovery) in a vaccine toxicology study, there are a total of 20/gender/group. |
| Would the use of a transgenic mouse be accepted to investigate breakage of tolerance to a self antigen? | Yes, a transgenic mouse expressing a human protein could be used to investigate breakage of tolerance to a self-antigen. However, depending on whether or not the human protein is expressed during T cell selection in the mouse, it is possible that it might be easier to break tolerance to the human protein in the mouse than it would be in humans. Another approach is to assess a mouse-mimic vaccine in a normal mouse model. |
| Should biodistribution studies be under GLP compliance? | Yes, biodistribution studies should be performed under GLP compliance. In some cases, the in-life portion of the study is GLP compliant and the assays are conducted non-GLP if it is not possible to perform the assays in a GLP manner. Exceptions to GLP compliance can be noted in the GLP compliance statement in the study report. |
| Could you elaborate more on: 1) How did you manage concern for T-cells attacking cells that express antigen on repeat dose. 2) How did you manage self-antigen recognition? Were there any tissues that were a no go if they expressed your target? | We used 3 approaches to understand the potential for T-cells attacking cells, that included understanding homology between vaccine and NHP antigen, understanding the expression level and distribution of the antigen in different tissues; lastly, understanding other proteins (and their distribution) that may be recognized by the T-cells. All of this helped to understand the potential for cross recognition of self-antigen. We did not have any predefined tissues that were a no go; it would also depend somewhat on the relative expression level in a tissue, compared to the expression on your target tissue, ie tumor. |
| Is there are need to conduct specific juvenile toxicity studies for therapeutic vaccines that may be used in children.  Are juvenile animal studies not required for prophylactic vaccine even if pediatric population is included in clinical trials and clinical use? | Juvenile toxicity studies are not typically performed for vaccines. It should be noted that depending on the age of the animals used in the toxicology study, at the study start, animals might be in the adolescent phase and mature into adults during the course of the study. Also, if DART studies are performed, the effect of the antibodies passed during gestation to the infant can be assessed in the post-natal phase of the study. Furthermore, in clinical trials, vaccine safety is first studied in healthy humans prior to stepping down in age to children. |
| For prostate cancer vaccine did you consider the use of a mouse surrogate for toxicity studies? | Our preference is always to use the clinical candidate if possible. In this instance we had high homology between the antigen and the NHP, we also had evidence that there was cross recognition of the target between the species, therefore, we decided it was appropriate to use the NHP. The use of a mouse surrogate while has some advantages with respect to sequence, it can always leave the questions as to the relevance to the clinical candidate. |
| Are there any therapeutic cancer vaccine specific guidances being developed? Or mRNA specific guidances? | EMA’s Guideline on the Evaluation of Anticancer Medicinal Products in Man is currently under revision, however, the current version only includes a couple of paragraphs on animal studies.  http://www.ema.europa.eu/docs/en\_GB/document\_library/Scientific\_guideline/2016/03/WC500203320.pdf  To our knowledge, there are no specific mRNA guidance documents being developed, however, existing guidelines cover considerations for all vaccine modalities. |
| For lipid nanoparticle delivery strategies, do these need to be assessed without the cargo, mRNA for example? | It depends on whether toxicology data with the lipid nanoparticle already exist and can be referenced. If a lipid nanoparticle alone control group would be helpful to interpret the results of the toxicology study, then it should be included. Also note that if the mRNA cargo modifies the structure and properties of the lipid nanoparticle, then a control group containing a lipid nanoparticle with a different (scrambled) mRNA might be more useful to include as a control group than an empty lipid nanoparticle. |
| Peak viral exposure at the injection site, why did you select Day 2 versus Day 1 or an earlier timepoint? | In toxicology studies the day of dosing is defined as Day 1, so a Day 2 time point is 24 hours after dose administration. A significant drop in viral copies at the site of injection can be observed between 24 and 48 hrs post dose if using an RNA-based viral vector. For DNA based vectors, there is unlikely to be a huge difference between 24 and 48 hr time points. In the example described, a replicating viral vector was used, and a 24 hr time point may also capture possible replication at the injection site. |
| Is the immunocompetency of the offspring tested as part of the DART studies for vaccines? | Unless there is a specific concern for immunosuppression induced by the vaccine, the immunocompetency of the offspring is not evaluated as part of the DART studies. |
| Can the biodistribution study be integrated into the repeated dose tox study | Yes, biodistribution evaluations can be integrated into the repeat-dose toxicity study. |
| Are cancer vaccines also reviewed by CBER? | Yes, CBER’s Office of Tissues and Advanced Therapies (OTAT) regulate cancer vaccines. |
| Vaccine tox use NHP as last resort...so I am interested in how you maximise data from discovery studies of regimen (immune response) optimization, to ensure maximum boosting occurs | Discovery pharmacology studies in NHPs (where the NHPs are not terminated and can be used again) are often used to optimize the vaccine dose, formulation and vaccination administration. After these pharmacology studies are performed, the toxicology studies can be performed in a different relevant species. As mentioned, NHPs are only used a last resort in toxicology studies and only if there is no other relevant species. |
| I recently transitioned to animal health. How do the requirements for safety in human vaccines compare to reqirements for animal vaccines? | In the US, animal vaccines are regulated by United States Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service’s (APHIS) Center for Veterinary Biologics (CVB). Safety evaluation of animal vaccines needs to be evaluated prior to licensure of the vaccine. This is codified in the Code of Federal Regulation Title 9, and several useful guidelines can be found at the following website:  https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/biologics-regulations-and-guidance/ct\_vb\_vs\_memos |
| Was a combination toxicity study needed to support administration of the cancer vaccine with a checkpoint inhibitor? If not, how was safety assessed? | This is somewhat on a case-by-case basis. For the anti-CTLA4 antibody, since we had data demonstrating that it increases the cellular immune response, we felt it important to include that antibody in all the toxicology studies. In the case of the anti-PD-1, since we were using a different route of administration, we included it primarily to assess local tolerability. Generally speaking, in non-tumor bearing animals, little toxicity has been seen with many of the checkpoint inhibitors. Since our toxicology studies are conducted in non-tumor bearing animals, there can likely be a justification for not necessarily including them in the toxicology studies, however this can be an area of discussion with regulators at pre-IND meeting, etc |
| In view of the fact that NOAEL is not used for FIH dose selection in oncology (for advanced cancer) why is it important to establish a NOaEL for a therapeutic cancer vaccine? Also, if the only effects observed are immune mediated, how is NOAEL set? | The NOAEL is important for therapeutic vaccines in general, especially for vaccines that may be indicated for a population that is still fairly healthy (e.g. a vaccine for smoking cessation). For advanced cancer patients, there are different considerations as mentioned in the question and the NOAEL determination is not as important. If the effects are only immune mediated and considered pharmacology, then this is not considered adverse, the NOAEL is the highest vaccine dose tested in the toxicology study. |
| Which division of FDA should be approached for seeking guidnace on prophylactic vaccine before embarking on tox studies ? | Assuming that the prophylactic vaccine is indicated for an infectious disease and that the vaccine is to be administered to humans, the FDA’s Center for Biologics Evaluation and Research’s (CBER) Office of Vaccines Review and Research (OVRR) should be consulted. |
| Why administering again recom protein/prime at the end of the cycle in DART studies? | It is typical to dose at the end of gestation to assess exposure to the vaccine late in gestation (ie - 3rd trimester timeframe in humans), and consistent with FDA guidance for vaccine DART studies. This ensures that developed fetuses are exposed in utero to the vaccine components in addition to the anti-vaccine antibodies. |
| If shedding was observed at the end of the study, how do you proceed with the program? | The development program proceeds with appropriate information about shedding and specific precautionary language to be included in the Investigator’s brochure and Informed Consent form for the clinical trial. If the vaccine is considered a genetically modified organism, then environmental precautions need to be taken. Eventually these precautions need to be included in the product label, if the vaccine makes it through development to licensure. |
| Can you comment on the likely regulatory expectation on the need for DART with subsequent antigen inserts for a plasmid backbone already evaluated in DART with a different antigen insert and no findings? | It depends on what the antigen insert is and whether or not the expressed antigen might have specific pharmacological effects that are a safety concern. If the insert encodes for a viral or bacterial pathogen that is not homologous to self-proteins, then a case could be made for not needing to repeat DART. Consultation with regulatory agencies is highly recommended when considering the omission of toxicology studies. |
| Do you think that vaccines could be used treat or prevent prion induced diseases? | Yes, it is possible that vaccines could treat or prevent prion-induced diseases and this concept has been gaining interest.  Reference: Burchell and Panegyres .Immunotargets Ther. 2016 Jun 16;5:57-68. doi: 10.2147/ITT.S64795. eCollection 2016. |
| about biodistribution, which tissue should be collected for QPCR? | At a minimum: blood,injection site(s) skin and muscle, gonads, brain, liver, kidneys, lung, heart, thymus, and spleen. Consider other tissues for evaluation, depending on the vector type and the transgene, as well as the route of administration (e.g., draining lymph nodes  and contralateral sites for subcutaneous/intramuscular injection, bone marrow, eyes, etc.)  Reference: FDA’s Guidance for Industry Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events (2006) |
| Case 2 Study: What additional (pharmacology and/or toxicology) studies may be required if the prostate cancer vaccine is proposed to be used in combination with immune checkpoint inhibitors? | This would need to be assessed based on knowledge of the pharmacology of the checkpoint inhibitor and the vaccine. It is necessary to provide data that justifies that the combination product is likely to provide greater efficacy, realizing that in vivo efficacy data may be challenging. Since much of the pharmacology and toxicology work may be conducted in non-tumor bearing animals, it is possible that in vivo combination studies might not be required prior to conducting clinical studies with additional immune checkpoint inhibitors. |
| what would be the tox plan differences for infectious disease prophylactic vaccine vs therapeutic? | Please see slides 11-23 in the presentation. Slide 23 has a summary that is helpful for repeat-dose toxicity study considerations. |
| What impact of Vaccine guidelines will be on ICH S5 R2 DART guidelines. | Male fertility studies are not usually performed for vaccines unless there is a cause for concern or a specific request from a regulatory agency to conduct such a study. Histopathology of male reproductive organs in the repeat-dose toxicity study can be used to assess potential effects. |
| Whether any suggestions to be made for Vaccine development in ICH s5R2 revision | Vaccines are included in ICH S5 R2 Revision and the recommendations are consistent with other vaccine guidelines. Please see Section 4.1.3 in the Step 2 version of ICH S5 R3.  http://www.ich.org/fileadmin/Public\_Web\_Site/ICH\_Products/Guidelines/Safety/S5/S5-R3EWG\_Step2\_Guideline\_2017\_0705.pdf |
| It was mentioned if the full human dose cannot be administered, the dose can be justified on a mg/kg basis. The full human DV can be a challenge in rats dosed IM, even when divided across sites. Is it common to justify the dose on a mg/kg basis in rats? | It is not very common to justify the dose on a mg/kg basis in rats, and instead a full-human dose is administered. For a 0.5mL IM vaccine dose, it is possible to divide this into two 0.25mL doses and administer in the quadriceps muscle. Depending on the rat strain used and age of the animal, it might not be feasible to administer this volume, in which case a maximum feasible dose (e.g. 0.1 mL or 0.2mL per quadriceps muscle) can be administered and justified accordingly. |
| Will the slides be available for download? | Yes, the slides are available for downloading together with the recording of the webinar. |
| Will slides be available for non-ACT members as well? | Slides can be made available upon request to ACT. |