In Vitro MABEL Approach for Nonclinical Safety Assessment of MEDI-565 (MT111)

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Summary

MEDI-565 (MT111) is a novel bispecific single-chain antibody of the BiTE® (bispecific T cell engager) class that transiently links carcinoembryonic antigen (CEA; also called CEACAM5, CD66e) on cancer cells with CD3 on T cells. Binding of MEDI-565 to CEA and CD3 results in T cell-mediated killing of cancer cells expressing CEA. MEDI-565 specifically binds to human and cynomolgus monkey CEA with high affinity but not to any other member of the CEACAM family; rodents do not express CEA. MEDI-565 binds to human CD3 but does not bind to cynomolgus monkey or mouse CD3. Consequently, no pharmacologically relevant animal species exists for testing the toxicity of MEDI-565. In an effort to introduce a pharmacologically relevant model, two surrogate antibodies were made, cyS111 and hyS111, with specificity to monkey or mouse CD3, respectively. However, the characteristics of these two antibodies were different from those of MEDI-565 to an extent that it was determined that hyS111 and cyS111 would not have utility in nonclinical toxicity studies. Hence, no in vivo toxicology studies were conducted in a relevant animal model with either MEDI-565 or the two surrogate antibodies. Rather, MedImmune implemented a strategy that utilized an in vitro approach to assess nonclinical safety instead of performing in vivo toxicity studies that would have required the use of nonhuman primates. Results from these studies were used to select an appropriate starting dose for Phase 1 clinical studies of MEDI-565 for the treatment of patients with cancers expressing CEA.

Keywords: in vitro toxicity study, monoclonal antibodies, biologics, carcinoembryonic antigen

Biotechnology-derived therapies (i.e., biologics such as antibodies) are products created by living organisms (bacterial, yeast, or mammalian cells) using recombinant DNA methods, and they encompass a wide range of molecular entities (Cavagnaro, 2002). Monoclonal antibodies are the most common type of biologic. Compared to small molecule drugs, biologics are more complex and may pose unique challenges in nonclinical safety assessment (Dixit et al., 2010). Nevertheless, the aims of nonclinical safety evaluations of biologics are similar to those of small molecules: to predict the likelihood of toxicity in humans, to identify target organs of toxicity, and to investigate reversibility of any toxic effects. Other objectives of nonclinical studies are to provide information on mechanism of action and to investigate the fate of the molecule. Finally, results of nonclinical studies are used to recommend an appropriate starting dose for Phase I studies and to identify safety parameters for clinical monitoring. The overarching objective is to assure that the investigational drug product can be used safely in early clinical trials.

Selection of an appropriate pharmacologically relevant species is an aspect of particular importance in the design of a preclinical toxicology program for biologics. Pharmacological relevance can be based on a variety of parameters, such as target sequence homology, expression of receptor or epitope, tissue cross-reactivity, and binding affinity – with the caveat that, while binding is essential for molecules to act, it is not sufficient to demonstrate pharmacological action. Therefore, additional experimental data is required, such as *in vitro* bioactivity in the animal species versus human, and/or pharmacological activity *in vivo*.

In March 2006, serious, life-threatening adverse effects were reported following the administration of TGN1412, a novel monoclonal antibody, to healthy volunteers (Horvath and Milton, 2009). The outcome of this tragic incident and the subsequent investigations was a report on Phase 1 Clinical Trials, written by an expert scientific group appointed by the Medicines and Healthcare Products Regulatory Agency (MHRA) and led by Sir Gordon Duff (Expert Group on Phase 1 Clinical Trials). This report, known as the Duff Report, provided recommendations and considerations for the authorization of such clinical trials in the future. In particular, it described what may be necessary to consider in the transition from preclinical to first-in-human

Phase 1 studies for biologics with novel mechanisms of action, agents with highly species-specific action, or agents directed towards immune system targets. A broader approach to dose justification that utilizes all available information was endorsed. Emphasis on pharmacological activity, in addition to more traditional toxicity-based algorithms, should be considered. The minimal effective dose in the pharmacological dose-response, as well as the range of doses that might produce unacceptable toxicity, such as the no-observed-adverse-effect-level (NOAEL) in the toxicological dose-response, should be considered. One method proposed was to determine the minimum anticipated biological effect level (MABEL) prior to determining a starting dose for first-in-human clinical studies.

MEDI-565 (MT111) is a novel bispecific single-chain antibody of the BiTE® (bispecific T cell engager) class that transiently links carcinoembryonic antigen (CEA; also called CEACAM5, CD66e) on cancer cells with CD3 on T cells. Carcinoembryonic antigen is a well characterized tumor-associated antigen that is expressed at low levels in normal tissues of epithelial origin (Hammarström, 1999) and is frequently over-expressed in carcinomas, including colorectal, gastric, lung, breast, pancreas, and ovarian cancer (Hammarström, 1999; Sanders et al., 1994). Cancer cells not only lose polarized (luminal) expression of CEA but actively cleave CEA from their surface by phospholipases, an action that results in high serum levels of CEA (Hammarström, 1999).

Binding of MEDI-565 to CEA and CD3 results in T cell-mediated killing of cancer cells expressing CEA (Lutterbuese et al., 2009). MEDI-565 specifically binds to human and cynomolgus monkey CEA with high affinity but not to any other member of the CEACAM family; rodents do not express CEA. MEDI-565 binds to human CD3 but does not bind to CD3 of cynomolgus monkey or mouse. Consequently, no pharmacologically relevant animal species exists for testing the toxicity of MEDI-565.

In an effort to introduce a pharmacologically relevant model, two surrogate antibodies were made, cyS111 and hyS111, with specificity to monkey or mouse CD3, respectively. Each of these surrogate molecules utilizes the anti-CEA binding arm of MEDI-565 combined with the cognate species-specific anti-CD3 binding arm for cynomolgus monkeys (cyS111) or mice (hyS111). That is, cyS111 binds to cynomolgus monkey CD3 and human CEA and cross-reacts with cynomolgus monkey CEA; hyS111 binds to mouse CD3 and human CEA, and since there is no mouse ortholog of CEA, studies with hyS111 must be performed using a mouse strain transgenic for human CEA.

The binding affinity and *in vitro* pharmacodynamic effects of MEDI-565 were compared to those of hyS111 and cyS111 using analogous model systems; results were used to determine the potential utility of these surrogate BiTE® molecules to predict the human toxicity of MEDI-565. The results of these studies revealed nonspecific activity and different functional characteristics for these surrogates compared to MEDI-565. For example, hyS111 differed from MEDI-565 in its affinity for its respective CD3 subunit of the T cell receptor, its *in vitro* potency, kinetics, and magnitude of T cell activation, and its ability *in vitro* and *in vivo* to induce T cell activation in the absence of human CEA on target cells. The *in vitro* pharmacology of the cynomolgus surrogate, cyS111, and MEDI-565 were compared to evaluate the potential use of cyS111 in a nonhuman primate animal species model for prediction of the human toxicity of MEDI-565. MEDI-565 and cyS111 differed in their affinities to their respective CD3 subunits. MEDI-565 activated T cells only in the presence of CEA. In contrast, cyS111 nonspecifically activated T cells in the absence of CEA.

It was concluded that the nonspecific activities (T cell activation independent of CEA binding) of both surrogates likely would misrepresent the specific activity and effects of MEDI-565 in humans, thereby limiting their utility in nonclinical toxicity studies. For this reason, a nonclinical strategy was implemented without using hyS111 or cyS111, and no *in vivo* toxicology studies were conducted in a relevant animal model with either MEDI-565 or the two surrogate antibodies. Rather, MedImmune implemented a strategy that utilized an *in vitro* approach to assess nonclinical safety instead of performing *in vivo* toxicity studies. The nonclinical safety of MEDI-565 was assessed in a cell-based system using co-cultures of human PBMC and CEA-positive target cells to establish a dose response for activity. The most sensitive measure to achieve 20% maximal effect (EC_{20}) for cytokine release, lysis, T cell activation, proliferation, or receptor occupancy was identified to determine a MABEL. Results of the studies demonstrated the ability of MEDI-565 to induce T cell proliferation, and cytokine release required engagement of both CD3 on T cells and CEA on target cells. Furthermore, MEDI-565-induced lysis of tumor cells was determined to be the most sensitive measure of MABEL.

A nonterminal pharmacokinetic (PK) study was performed in six male cynomolgus monkeys to establish exposure parameters following a single IV or a single subcutaneous (SC) dose of MEDI-565 using a crossover design. Results demonstrated a large volume of distribution, high SC bioavailability, and long terminal elimination half-life compared to similar BiTE® molecules. Human PK parameters were predicted from cynomolgus monkey PK parameters using allometric scaling to determine a human dose that would result in exposures around the identified MABEL concentration. Results from these studies were used to select an appropriate starting dose for Phase 1 clinical studies of MEDI-565 for the treatment of patients with cancers expressing CEA.

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