An FDA oncology analysis of immune activating products and first-in-human dose selection

Haleh Saber*, Ramadevi Gudi, Michael Manning, Emily Wearne, John K. Leighton

US Food and Drug Administration, Center for Drug Evaluation and Research, Office of Hematology and Oncology Products, 10903 New Hampshire Ave, Silver Spring, MD 20903, United States

ABSTRACT

As sub-therapeutic doses are not medically justifiable in patients with cancer, we retrospectively analyzed data on immune activating products, to assess approaches used in first-in-human (FIH) dose selection, the utility of animal toxicology studies in dose selection, and the length of time to complete FIH trials. The information collected included pharmacology and toxicology data, FIH dose and rationale, and dose-finding trial design. We used the principles of the Hill equation to estimate the FIH doses for antibodies and compared them to the doses administered to patients with acceptable toxicities. For approximately half the antibodies (44%) examined, the FIH doses were at least a hundred-fold lower than the doses safely administered to patients, indicating optimization of FIH dose selection and/or optimization of dose-finding trial design is needed to minimize patient exposure to sub-therapeutic doses.

However, selection of the FIH dose for antibodies based on animal toxicology studies using 1/6th the HNSTD or 1/10th the NOAEL resulted in human doses that were unsafe for several antibodies examined. We also concluded that antibodies with Fc-modifications for increased effector function may be less tolerated, resulting in toxicities at lower doses than those without such modifications. There was insufficient information to evaluate CD3 bispecific products.

1. Introduction

Advances in science and a better understanding of mechanisms of tumor progression have led to innovative medicines for the treatment of cancer. Immune oncology (IO) pharmaceuticals are among these innovative products and activate the body’s immune system against tumor cells, e.g. by activating T-lymphocytes and antigen-presenting cells (Couzin-Frankel, 2013; Mellman et al., 2011). The field of IO pharmaceuticals has been growing rapidly, with interest spurred by the recent approvals of the PD-1 inhibitors Keytruda and Opdivo, and the CD3 bispecific construct Blincyto. While the science is advancing rapidly, there are regulatory areas to be addressed, specifically regarding the safety assessment of IO pharmaceuticals prior to initiation of clinical studies and first-in-human (FIH) dose selection (Muller and Brennan, 2009; Vatsan et al., 2013).

In 2006, administration of the anti-CD28 monoclonal antibody (mAb) TGN1412 to healthy volunteers resulted in multi-organ dysfunction in six subjects due to cytokine storm; its adverse effects occurred within 90 min of a single infusion (Suntharalingam et al., 2006). Subsequent to the TGN1412 tragedy, cautionary approaches to FIH dose selection have been proposed for products that may activate the immune system (EMA, 2007; EMA, 2013; ICH S9, 2010). As a result, a conservative method to select the FIH start dose was proposed for agonists based on the minimally-anticipated biological effect level (MABEL) that results in no more than 10% receptor occupancy (RO).

Various approaches for estimating RO or selecting a dose based on a desired RO have been described (Goutelle et al., 2008; Lowe et al., 2007; Muller and Brennan, 2009); one approach is based...
on the Hill equation (Goutelle et al., 2008), which we used in our analysis for FIH dose estimation. The Hill equation assumes that the concentration of the drug is much greater than the concentration of the receptor. Other approaches that take into account the number of receptors, clearance, or the receptor turnover rate may be used when such data are available; the FIH dose obtained using these factors is anticipated to be higher than by using solely the equations below. These factors were not available for sufficient number of products for cross-IND comparison. The following Hill- and Michaelis-Menten- based equations were used for FIH dose estimation, based on in vitro activity data (A) or binding constants (B):

\[
PA = \frac{|C|}{|EC_{50} + |C|} \quad (A)
\]

\[
RO = \frac{|C|}{K_D + |C|} \quad (B)
\]

C is the human plasma concentration of the biopharmaceutical, PA is the pharmacologic activity, RO is the receptor occupancy, EC50 refers to human values obtained from in vitro activity (i.e. not binding) data and K_D is the human antigen dissociation constant. For the purpose of our analysis, we used a Hill coefficient of 1 (Goutelle et al., 2008) due to lack of data to estimate the coefficient. A coefficient of 1 will result in a more conservative FIH dose compared to doses obtained when the coefficient is > 1.

One objective of nonclinical studies is to identify a safe FIH dose that is sufficiently high to minimize exposure to sub-therapeutic doses in serious and life-threatening diseases, such as metastatic cancer, and to allow rapid attainment of the recommended Phase 2 dose (RP2D). The FIH dose selection for small molecules (1/100th STD_10 or 1/6th HNSTD) is well established and discussed in ICH S9. Recently we also examined acceptable approaches to dose selection for the first generation antibody-drug conjugates (Saber and Leighton, 2015). The approach to FIH dose selection for biological products in oncology is also discussed in ICH S9, including the MABEL approach for biopharmaceuticals with immune agonistic properties.

We have conducted a retrospective analysis of nonclinical development programs for immune checkpoint inhibitors and stimulators and other antibodies with the potential to activate the immune system, and CD3 bispecific constructs. We reviewed the nonclinical development programs submitted to support 32 separate investigational new drug applications (INDs), including pharmacology and animal toxicity studies, and initial dose-finding trial designs with an emphasis on FIH dose selection. Some of the products examined are now FDA-approved.

2. Methods

The FDA archival database was searched for the keyword “MABEL” to identify anticancer products that used a MABEL approach for setting the FIH dose; this search identified over 100 potential INDs. This search also identified some INDs in which the MABEL approach was discussed but not used to set the FIH dose, e.g., when a side-by-side comparison of the investigational IO product and an IO product in clinical trials was conducted to allow a higher FIH dose than supported by nonclinical data. The INDs were then screened for antibodies and bispecific antibody constructs for which a sufficient number of patients received the drug to identify a maximum tolerated dose (MTD), an optimal biologic dose (OBD), or a recommended human dose for further investigation, and for those still dose escalating, to have dose-limiting toxicities (DLTs), have multiple cohorts completed in the absence of DLTs, or to have reached receptor saturation. Thirty-two INDs were identified and included in our analysis.

2.1. Data collected

When available, the following information was collected for each IND: date of IND submission; product characteristics (molecular weight, target antigen, IgG subtype, modifications to the mAb for modified effector function); in vitro activity studies conducted and corresponding EC50s; in vitro antigen binding data and corresponding dissociation constant (K_D); non-human primate (NHP) toxicology data; the sponsor’s approach to setting the FIH dose; highest human doses (HHDs) with acceptable safety profile (based on Investigator’s Brochure, annual reports, or safety reports at the cut-off date of July 15, 2016); human MTD, OBD, or the recommended human dose (label dose for approved drugs); dose-finding clinical trial design (single patient versus 3 + 3 design; intra-patient versus inter-patient dose escalation, dose increments); monitoring and treatment for infusion-related reactions (IRRs)/ cytokine release syndrome (CRS). In this article we refer to infusion reactions and antigen binding-associated cytokine release as IRR/ CRS as symptoms overlap (Brennan et al., 2010; Doesselgger and Banholzer, 2015) and the terms were at times used interchangeably in INDs.

2.2. Product characteristics

In this analysis, we only included products with the potential to activate the immune system, directly or indirectly. During screening, products were selected based on common knowledge of target involvement in immune activation (e.g., checkpoint inhibitors and stimulators), or based on data presented in the IND or a literature search suggesting the potential involvement of the target in immune activation.

Of the 32 INDs in our database, five were CD3 bispecific constructs, one was an IgG4 trimeric antibody, and 26 were monoclonal antibodies of IgG1 (18 products), IgG2 (3 products) or IgG4 (5 products) isotype. The targets of antibodies in our dataset include, but are not limited to: PD1, PD-L1, CD40, GITR, OX40, OX40L, CD33, CD38, CD19, CD137 (4-1BB), c-fms, B7 family member antigen, and CTLA-4. Products targeting other antigens were also included in this analysis but are not specifically identified in this article.

2.3. FIH dose computation

Due to low number of CD3 bispecific constructs, structural heterogeneity, and schedule differences in administration, these products have been excluded from FIH dose computation.

We utilized the principles of Hill equation as described in the Introduction, with a plasma volume of 2.8 L, and data from in vitro assays, to calculate a FIH dose that would result in 20%–80% RO or 20%–80% PA. We chose 20%–80% range for the RO and PA because 20% is currently the most common occupancy and activity level used for FIH dose selection (by sponsors and by FDA/OHOP), and 80% is below the RO that resulted in cytokine storm with TGN1412 (TGN1412 was at 90% RO at the FIH dose).

In our analysis, only K_D was used for FIH dose computation at the pre-defined RO of 20%–80% (equation B); binding EC50s were not used since cell-based assays are associated with variability (e.g. based on the cell line used and the expression level of antigen). Multiple approaches could be used to estimate the FIH dose based on RO and PA; however, the decision to use the Hill equation was based on the fact that it is commonly used by sponsors and reviewers of INDs and binding and activity data were available for most INDs. This allowed for estimation of the dose and for cross IND examination. Moreover, as binding data is available for TGN1412,
results obtained from our analysis could be compared to that of TGN1412. Approaches based on xenograft studies were not assessed, because xenograft studies were not always conducted, and when conducted, the condition of the studies varied greatly (clinical candidate versus mouse surrogate) or the relevance of findings to humans was not clear (e.g. while the antigen binding may occur, the FcR binding may not occur or may not be relevant). Analysis of the MABEL approach based on expression level of antigen in tumor cells (Lowe et al., 2007) was not conducted, because the information was not provided for most INDs.

In addition to RO and PA, we examined FIH doses obtained based on 1/10th NOAEL and 1/6th HNSTD from NHP toxicology studies, using body weight (BW) and body surface area (BSA) for animal-to-human dose conversion.

2.4. Binding and activity data

Binding and activity data were obtained mainly from nonclinical summaries provided by sponsors and FDA pharmacology/toxicology reviews. Study reports were occasionally examined to answer specific questions. The range of activity EC50 and Kd values were captured when available. Not all INDs included the EC50 values even when it appeared this data could have been obtained. The FIH dose using the activity data was computed only when EC50 values were provided; an estimation of EC50 values from the graphs (when included in the IND) was not conducted as the raw data were not available. The same approach was taken for FIH dose estimation using the binding data, Kd. Kd values refer to antigen binding dissociation constant. Dissociation constants for FcR binding were not collected; regardless, these values were available for a limited number of applications only, e.g. when the Fc domain was modified.

3. Results

3.1. CD3 bispecific constructs

Of five CD3 bispecific constructs in our database, three products were of relatively low molecular weight (50–105 kDa) and were administered to patients daily; two products contained Fc domains and were administered weekly due to longer half-lives. All bispecific constructs were administered by slow IV infusion (generally over 2–4 h in the first cycle) or continuous IV infusion; in one IND, slow IV infusion was replaced by continuous IV infusion after IRRs/CRS were observed. Only one bispecific construct has been administered to patients at relatively high doses (60–90 mg weekly), while the rest were dosed in the microgram or low milligram range. For several products, there appeared to be difficulties in escalating the doses in the dose-finding trials, requiring protocol amendments to address the safety issues. Protocol amendments included increasing the duration of infusion, reducing the frequency of administration, and including additional measures for IRR/CRS treatment. Table 1 provides information on product characteristics; higher Kd values indicate lower affinity.

2.6. Antibody Fc effector function

Seven IgG1 antibodies in our database were modified in the Fc domain, which can impact the effector function. Of the antibodies examined, a CD19 IgG1 and a CD40 IgG1 antibody were afucosylated for increased ADCC activity. Another CD40 IgG1 antibody was modified in the Fc domain and, compared to the antibody with wild-type IgG1 Fc domain, had reduced affinity towards FcyRIIIA (CD16A) and enhanced binding towards FcyRIIB (CD32B). A checkpoint inhibitor targeting a B7 family antigen had an Fc-optimized region for increased affinity towards CD16A and decreased affinity for CD32B. An IgG1 antibody against CD33 was modified in the Fc domain for increased binding affinity towards CD16A and as expected, this resulted in increased ADCC activity compared to a non-Fc-engineered IgG1-type of the antibody. An IgG1 antibody against PD-L1 and an IgG1 antibody against GITR were nonglycosylated to prevent Fc-mediated effector function. Modifications in other antibodies (if any) were not for increased/ decreased effector function; for instance, modifications in the Fc domain to avoid arm exchange for IgG4 isotype antibodies.

For IgG4 antibodies targeting PD-1, doses up to 10 mg/kg (Q2W or Q3W) have been administered to patients in the dose-finding trials without sequelae, understanding that these trials are rather short in duration and limited in the number of patients exposed to the pharmaceutical, and may not necessarily predict the full safety profile. It remains to be determined whether for unmodified antibodies of the same isotype targeting the same antigen with comparable affinity, the HHD with acceptable toxicities may be in the same range. Modifications to the antibodies may change the potency and impact human MTDs. Two IgG1 antibodies in our database targeting CD40 (checkpoint stimulators) were administered to patients every-3-weeks (referred to as Antibodies A and B). Antibody A was afucosylated and Antibody B had reduced binding to FcyRIIIA (CD16A) and enhanced binding to FcyRIIB (CD32B). Modifications in the Fc domain of the two antibodies result in effector

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Bispecific constructs.</strong></td>
</tr>
<tr>
<td>Product</td>
</tr>
<tr>
<td>CD3-CEA</td>
</tr>
<tr>
<td>With Fc domain</td>
</tr>
<tr>
<td>MW &gt; 150 kDa</td>
</tr>
<tr>
<td>CD3-CD19 (Blinycyo)</td>
</tr>
<tr>
<td>No Fc domain</td>
</tr>
<tr>
<td>MW &gt; 54 kDa</td>
</tr>
<tr>
<td>CD3-CEA</td>
</tr>
<tr>
<td>No Fc domain</td>
</tr>
<tr>
<td>50 &lt; MW &lt; 105 kDa</td>
</tr>
<tr>
<td>CD3-CD19</td>
</tr>
<tr>
<td>No Fc domain</td>
</tr>
<tr>
<td>50 &lt; MW &lt; 105 kDa</td>
</tr>
<tr>
<td>CD3-PSMA</td>
</tr>
<tr>
<td>With Fc domain</td>
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<tr>
<td>MW &gt; 150 kDa</td>
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</table>

* Highest human dose from clinical trials except for the approved product, Blinycyo, for which the label dose is included.

* Based on binding EC50s.
function that would be different between the two antibodies. Antibody A resulted in Grade 4 IRR/CRS at a dose of 0.06 mg/kg (3.6 mg for a 60 kg subject) that corresponds to approximately 90% RO. Antibody B resulted in Grade 4 IRR/CRS at a dose of 1 mg/kg (60 mg for a 60 kg subject), above saturation. The difference in the tolerated doses is not a result of antigen-binding; a cross IND comparison indicates lower affinity of Antibody A against CD40 compared to Antibody B and hence predicting better tolerance for antibody A.

A CD33 IgG1 antibody with increased CD16A binding affinity was administered weekly to patients at a FIH dose of 10 mg that corresponded to 90%–95% RO. The FIH dose was associated with Grade 3 toxicities that resulted in a protocol amendment to include additional monitoring for hepatotoxicity and treatment for IRR/CRS. Although the initial clinical trial contained measures to address IRR/CRS, additional measures were added that included reduced infusion rate. With these modifications, the sponsor was able to administer the 10 mg dose to additional patients, for a total of 32 subjects at the same dose level as of the cut-off date for this analysis of July 15, 2016.

3.3. In vitro activity data

In vitro activity studies for all products (antibodies and bispecifics) pertinent to immune system activation included the following assays: cytokine release, T-cell proliferation, T-cell activation markers (e.g. CD69 and CD25), target cell lysis or apoptosis, and ADCC/CDC as appropriate. While most INDs contained multiple in vitro activity studies, occasionally INDs contained no or minimal evaluation (e.g. only one activity study). Results of the studies conducted, and at times literature assessment, contributed to hazard identification and hence the decision to use a conservative approach for FIH dose selection by the sponsor and/or the reviewer. No one type of study was the most sensitive in predicting a lower threshold for toxicity; e.g., while T-cell proliferation was the most sensitive assay for one product, cell lysis was the most sensitive for another product.

When multiple in vitro activity assays were conducted and assays were optimized, the range of mean EC50s in these assays was wide. For instance, for one product (Table 2), the range was >10,000 fold for mean EC50s (largest-to-smallest). Not all sponsors provided the EC50s for assays conducted with positive outcome, therefore the range of EC50s were usually not available. EC50s varied greatly depending on the conditions of the assays, e.g. cell lines used, the incubation time, and effector-to-target cell ratio.

3.4. Cytokine release assay

Given the life-threatening cytokine storm that occurred in healthy volunteers administering a single dose of the anti-CD28 mAb TGN1412, most sponsors assessed in vitro cytokine release from whole human blood or human peripheral blood mononuclear cells (PBMCs) in order to investigate the potential for their products to induce systemic release of proinflammatory cytokines such as TNF-alfa, IL-2, IL-6, IL-10, and IFN- gamma from target immune cells in humans. Appropriate in vitro cytokine release assay (CRA) platforms have been explored and are discussed elsewhere (FDA, 2014; Römer et al., 2011; Stebbings et al., 2007, 2013). Ultimately, the selected CRA platform(s) should be relevant to the mechanism of action of the product (Grimaldi et al., 2016).

Prior to FIH dosing, stand-alone in vitro CRAs were conducted for 27 of 32 INDs (84%) included in our analysis. For one IND investigating an IgG4 mAb targeting PD-1, a stand-alone in vitro CRA was not conducted prior to initiation of the FIH study, but was conducted shortly after initiation of the FIH clinical trial for a total of 28 CRAs conducted before or shortly after initiation of the FIH trial. Of the four sponsors that did not conduct stand-alone CRAs, one sponsor evaluated in vivo cytokine release as part of the monkey toxicology study, but none of these sponsors assessed cytokine release as part of other in vitro activity assays. In general, sponsors used standard CRA platforms for hazard identification. Fifteen out of 28 CRAs (54%) utilized soluble and immobilized/plate-bound presentation of antibody, whereas 12 out of 28 CRAs (43%) utilized either soluble or immobilized presentation, the majority of these being soluble. Information regarding the assay platform was not available for 1 out of 28 (4%) of the INDs. Negative control(s) including but not limited to medium alone, phosphate buffered saline, isotype-matched negative control antibodies, and marketed antibodies known to not cause clinical cytokine release (e.g. cetuximab, trastuzumab, and bevacizumab) were routinely included in all of the CRAs. Known inducers of cytokine release including OKT3 (anti-CD3 antibody), alemtuzumab (anti-CD52 mAb), and TGN1412 (CD28 superagonist), lipopolysaccharide (LPS), phytohaemagglutinin (PHA), and PMA plus ionomycin were used as

### Table 2

Examples of activity assays, and range of EC50s and K<sub>D</sub>s.

<table>
<thead>
<tr>
<th>Product</th>
<th>Activity data</th>
<th>Range of EC50</th>
<th>Range of K&lt;sub&gt;D&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td>In vitro activity assay</td>
<td></td>
<td></td>
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<tr>
<td>CD3 bispecific</td>
<td>T-cell activation</td>
<td>200 ± 36 pg/mL (CD25)</td>
<td>Experiment 1 CD3: 260 nM Antigen: 1.5 nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>111 ± 33 pg/mL (CD69)</td>
<td>Experiment 2 CD3: 55.5 nM Antigen: 1.8 nM</td>
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<tr>
<td></td>
<td>Cytotoxicity/cell lysis</td>
<td>15–462 pg/mL</td>
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<td></td>
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<td>20–414 pg/mL</td>
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<td></td>
<td></td>
<td>1.6 ± 0.5 pg/mL</td>
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<td></td>
<td></td>
<td>0.3 ± 0.2 pg/mL</td>
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<tr>
<td></td>
<td></td>
<td>56.5 ± 21.6 pg/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Target cell depletion</td>
<td>80 ± 39 pg/mL</td>
<td></td>
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<tr>
<td></td>
<td>CRA: IL2</td>
<td>320 ± 138 pg/mL to 396 ± 178 pg/mL</td>
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<tr>
<td></td>
<td></td>
<td>497 ± 382 pg/mL to 1221 ± 1203 pg/mL</td>
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<tr>
<td></td>
<td></td>
<td>1743 ± 1420 pg/mL to 2193 ± 1607 pg/mL</td>
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<tr>
<td></td>
<td></td>
<td>2025 ± 1434 pg/mL to 2522 ± 337 pg/mL</td>
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<tr>
<td></td>
<td></td>
<td>836 ± 469 pg/mL to 1244 ± 614 pg/mL</td>
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<tr>
<td></td>
<td></td>
<td>2178 ± 1618 pg/mL to 4078 ± 2265 pg/mL</td>
<td></td>
</tr>
<tr>
<td>Checkpoint stimulator</td>
<td>Cytokine release assay (soluble/positive),</td>
<td>No EC50 provided</td>
<td>K&lt;sub&gt;D&lt;/sub&gt; = 1.2 nM</td>
</tr>
<tr>
<td></td>
<td>APC activation (positive), ADCC (positive)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CRA: Cytokine release assay. If only one EC50 or one K<sub>D</sub> was reported, it was assumed that it represents the mean value for that study.
positive controls in 24 out of 28 (86%) of the INDs. The results from CRAs were generally considered negative if the results were comparable with the negative control, typically medium alone or isotype control antibody.

3.5. In vitro binding data

Antigen-specific dissociation constant ($K_D$) values were determined by one or more of the following methodologies: flow cytometry, surface plasmon resonance (SPR), ELISA, kinetic exclusion assay (KinExA), and cell-based equilibrium binding assay. Even when multiple techniques were used $K_D$ values were in a tight range compared to activity EC50s with up to 20 fold differences (highest-to-lowest $K_D$ ratio). The range was tighter when SPR or kinetic exclusion assay was used to determine the $K_D$ (up to 6 fold). The range was wider when cell based assays were used for $K_D$ determination (up to 10 fold) or when multiple techniques were employed to assess $K_D$. For an IND where three separate methods were used to estimate $K_D$ (SPR, ELISA, and flow cytometry), the range was 20 fold. The tight range for SPR and KinExA may be due to defined conditions of use by each manufacturer as described in their manuals.

3.6. Animal toxicology studies

For most antibodies (25 out of 27, 93%), the cynomolgus monkey was selected as the pharmacologically relevant species for testing in the repeat dose toxicology studies conducted to support the FIH dosing. Out of the 27 antibodies, 1 (4%) used the chimpanzee as the toxicology species (not discussed further) and 1 (4%) did not conduct any toxicology studies due to the lack of a pharmacologically relevant species. The studies ranged from a single-dose administration to repeat doses of 1- to 3-month duration with a 4-8 week recovery period. Except for two antibodies that resulted in mortality in animals, other antibodies were well tolerated and the high dose was defined as the NOAEL or the HNSTD. Findings in animals were generally consistent with the mechanism of action of the antibodies. The toxicities and severities observed were not always dose dependent. Anti-drug antibodies were detected for some products and were associated with decreased exposure in some instances. There were no new toxicities observed in the recovery periods. Adverse effects of immune checkpoint stimulators and inhibitors (e.g. antibodies against CD40, OX40, GITR, B7 family, CTLA4, PD-1, PD-L1) included multi-organ inflammatory response; e.g. in kidneys, heart and eyes. Some INDs included measurements of cytokines production in animals; when included cytokine upregulation was not always detected and did not always correspond to the results of in vitro cytokine release results obtained using human cells.

Of the 5 CD3 bispecific constructs, only one contained a toxicology study in the monkey. For this IND, doses up to 10 mg/kg were administered to cynomolgus monkeys twice per week by IV bolus. The high dose was considered the HNTSD mainly due to decreased activity and vomiting. For one CD3 bispecific construct, the toxicity study was conducted in chimpanzees.

3.7. Sponsors’ rationale for FIH dose selection

A variety of MABEL approaches were used by sponsors to set FIH doses, employing any of the following data (20% is an example; slightly lower and higher values have been also proposed):

1. In vitro activity data: using the EC20 directly or using the Hill equation (and EC50) to determine a dose at 20% PA (with or without PK modeling).
2. In vitro binding data: using the antigen-specific $K_D$ to estimate the plasma concentration at 20% RO (with or without PK modeling).
3. Mouse xenograft data: the lowest dose that results in antitumor activity, with or without a safety margin.
4. Other methods: target expression in humans and PK modeling; undefined approaches.

When the target was not novel, some sponsors conducted a side-by-side comparison of their product to an approved product or to one or more products in clinical development; this strategy resulted in FIH doses substantially higher than those based on a MABEL approach.

3.8. OHOP’s analysis of FIH doses for antibodies

CD3 bispecific constructs were excluded as mentioned under methods.

As previously mentioned, we employed two methods for computing the FIH dose; FIH doses based on 20%–80% PA, and those based on 20%–80% RO. The FIH doses computed based on 20%–80% PA had acceptable toxicities for all antibodies examined, which included checkpoint inhibitors and stimulators. For the purpose of this research, “acceptable toxicities” refers to no drug-related death, no CRS/IRR greater than Grade 3 (per the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE)), and overall manageable toxicities. The range of potential FIH doses was large for a specific product when EC50s had a large range. FIH doses based on 20%–80% RO also had acceptable toxicities for all antibodies examined. Doses above saturation also had acceptable toxicities, with the exception of two Fc-modified antibodies with increased ADCC activity. For these two antibodies, toxicities at doses corresponding to ≥90% RO caused adverse findings that included IRR/CRS resulting in protocol amendments; see above under “Antibody Fc effector function.” When activity assays were optimized and the lowest activity EC50 was used, in general FIH doses based on activity studies resulted in lower FIH doses compared to doses based on occupancy for the corresponding percent PA and RO (e.g. FIH doses at 20% PA compared to those at 20% RO).

We also assessed whether 1/6th the HNSTD or 1/10th the NOAEL, using either body weight or body surface area for animal-to-human conversion, could be used to select the FIH dose. For several INDs, the predicted FIH doses were either above the human MTD or the HHD, or the doses were at the human MTD such that any dose escalation would result in unacceptable toxicities. Two examples are presented in Table 3; the animal toxicology studies in this table are GLP-compliant studies submitted with the initial IND. No repeat-dose toxicology studies were available for the antibody in Example 1 at the cut-off date of July 15, 2016.

3.9. First-in-human, dose-finding trial designs (antibodies)

Products were administered IV, generally with an infusion duration of 0.5–6 h in the FIH clinical trial. Common features in FIH, dose-finding trials included staggered dosing to include adequate duration of observation before the next patient was dosed. In addition, all INDs included measures to monitor for and treat IRR/CRS. Most INDs proposed to treat patients after the onset of IRR/CRS, while others proposed prophylactic treatment. Nearly half of the INDs had a FIH dose that was below 50% RO, requiring many dose escalation steps. While the low FIH doses may have contributed to the long dose escalation period of up to 5 years (Table 4AA), other factors such as slow recruitment were not examined and could not be excluded. In the INDs where the FIH
Table 3
FIH dose selection using 1/10th NOAEL or 1/6th HNSTD.

**Example 1: IgG1 against CD40**
The HHD of 0.06 mg/kg (~3.6 mg) resulted in one Grade 4 IRR/CRS High dose in monkeys – 30 mg/kg (HNSTD due to ocular inflammation); single dose 1/6th HNSTD using BW for conversion: HED = 5 mg/kg (300 mg)/1/6th HNSTD using BSA for conversion: HED = 96 mg

**Example 2: IgG1 against CD40**
The HHD of 1 mg/kg (~60 mg) resulted in one Grade 4 IRR/CRS High dose in monkeys – 30 mg/kg (NOAEL); weekly (5 total doses) 1/10th NOAEL using BW for conversion – 3 mg/kg (180 mg)/1/10th NOAEL using BSA for conversion: HED = 58 mg

HED: human equivalent dose.

Table 4A
Examples of antibodies with FIH doses at \( \leq 50\% \) RO.

<table>
<thead>
<tr>
<th>Target or class [date of IND submission]</th>
<th>FIH dose</th>
<th>HHD (time from IND submission to this dose)</th>
<th>RO* at HHD</th>
<th>Ratio of HHD to FIH dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Checkpoint stimulator [2014]</td>
<td>200 mcg</td>
<td>1200 mg (2 yr)</td>
<td>Saturated</td>
<td>6000</td>
</tr>
<tr>
<td>Checkpoint stimulator [2014]</td>
<td>36 mcg</td>
<td>3.6 mg (1 yr)</td>
<td>90%</td>
<td>100</td>
</tr>
<tr>
<td>Checkpoint stimulator [2015]</td>
<td>6 mcg</td>
<td>18 mg (1 yr)</td>
<td>Saturated</td>
<td>3000</td>
</tr>
<tr>
<td>Checkpoint stimulator [2014]</td>
<td>1.5 mcg</td>
<td>30 mg (1 yr)</td>
<td>Saturated</td>
<td>20,000</td>
</tr>
<tr>
<td>Checkpoint stimulator [2010]</td>
<td>6 mcg</td>
<td>120 mg (5 yr)</td>
<td>Saturated</td>
<td>20,000</td>
</tr>
<tr>
<td>Expected to activate the immune system based on experience with another product targeting the same antigen [2009]</td>
<td>6 mcg</td>
<td>1.2 g (5 yr)</td>
<td>Saturated</td>
<td>200,000</td>
</tr>
</tbody>
</table>

HHD: highest human dose with acceptable toxicities, at the cut-off date.
Note: doses are converted to a flat dose using 60 kg as the body weight.

HED: human equivalent dose.

Table 4B
Examples of antibodies with FIH doses at \( > 50\% \) RO.

<table>
<thead>
<tr>
<th>Target or class [date of IND submission]</th>
<th>FIH dose</th>
<th>HHD or RHD</th>
<th>Rationale for FIH dose selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Checkpoint stimulator [2014]</td>
<td>3 mg (saturated)</td>
<td>HHD: 600 mg</td>
<td>Based on another product in development</td>
</tr>
<tr>
<td>Checkpoint inhibitor [2010]</td>
<td>60 mg (saturated)</td>
<td>RHD: 120 mg</td>
<td>Based on another drug</td>
</tr>
<tr>
<td>Checkpoint inhibitor [2014]</td>
<td>60 mg (saturated)</td>
<td>HHD: 600 mg</td>
<td>Mainly based on other products inhibiting the same target</td>
</tr>
<tr>
<td>Checkpoint stimulator [2014]</td>
<td>600 mcg (60% RO)</td>
<td>HHD: 18 mg (saturated)</td>
<td>Based on other products in development</td>
</tr>
<tr>
<td>Checkpoint inhibitor [2011]</td>
<td>0.6 mg (80% RO)</td>
<td>RHD: 1200 mg (saturated)</td>
<td>FDA’s request for 15% RO and the sponsor’s counter proposal</td>
</tr>
</tbody>
</table>

HHD: highest human dose with acceptable toxicities, at the cut-off date.
RHD: recommended human dose; refers to the dose in the product labeling.
Note: doses are converted to a flat dose using 60 kg as the body weight.

Doses were above 50% RO (Table 4B), sponsors often relied on clinical experience with similar products in development. The differences between the FIH dose and HHD for all antibodies examined are shown in Fig. 1.

FIH protocols varied in the number of patients treated at low doses as well as the dose escalation scheme. Despite the low FIH doses, some protocols were 3 + 3 design. Others, however, proposed single patient cohorts or intra-patient dose escalations for the first few cohorts until a specific RO was obtained, receptor saturation reached, or certain grades of toxicities were observed. For some INDs when the FIH dose was low, the first few dose escalations were large, as much as 10 fold in between cohorts.

Below are examples from three INDs, depicting variations in FIH trial designs.

Example A describes an immune checkpoint inhibitor. The FIH dose of 0.01 mg/kg (weekly: 120-min IV infusion) was approximately 600 mcg using 60 kg as the body weight, and is at 10% RO using equation B (Table 5A). This dose was also 10-fold below the pharmacologically active dose (PAD) of 0.1 mg/kg in a xenograft study. Intra-patient dose escalation occurred up to a dose of 0.15 mg/kg (9 mg), at approximately 70% RO. At this point, 3 + 3 dose escalation began. The sponsor used half log dose increments throughout the trial, which is often used for therapeutic proteins. As of the cut-off date of July 15, 2016, doses up to 15 mg/kg (900 mg) weekly have been administered to patients; this dose has been expanded and defined as the MTD/MAD (maximum administered dose). The dose of 900 mg is above what is needed for receptor saturation. Toxicities observed with this antibody include IRR/CRS. According to the sponsor, “Events of infusion-related reaction and cytokine release syndrome have been managed with appropriate medical treatment and all have resolved without sequelae.” The time from the IND submission to 0.15 mg/kg dose (intra-patient dose escalation portion) was approximately one year. The time from IND submission to the MTD/MAD of 15 mg/kg (900 mg) was three years.

Example B describes an immune checkpoint stimulator. The FIH dose of 0.0001 mg/kg is approximately 6 mcg using 60 kg as the body weight and was administered every-3-weeks (Q3W) as a 60-min IV infusion (Table 5B). This dose is at 6% RO and 5000 fold below the PAD dose of 0.5 mg/kg in a xenograft study. The first two cohorts were single patients using one log dose increments. At the 3rd dose level of 0.01 mg/kg (600 mcg, receptor saturation) the sponsor switched to a 3 + 3 design using half log dosing increments. One patient exhibited Grade 4 IRR/CRS at the 1 mg/kg (60 mg) dose level. This patient had symptoms as early as 20 min into infusion. The dose of 1 mg/kg also resulted in Grade 4 thrombocytopenia. The dose has been since de-escalated to 0.6 mg/kg. The time from the IND submission to the 0.001 mg/kg dose at 55% RO (single patient cohort portion) was approximately one year. The time from IND submission to the human dose of 1 mg/kg...
(60 mg) was two years.

Example C describes another immune checkpoint stimulator. The FIH trial was a single dose administration of the product with a FIH dose of 0.0001 mg/kg, which is approximately 6 mcg using 60 kg as the body weight (Table 5C). Doses were administered as a single 60-min IV infusion. The FIH dose was at 2% RO. This dose was 40,000 fold below the PAD of 4 mg/kg in a xenograft study using a rodent homolog. Doses in patients ranged from 0.0001 mg/kg to 4 mg/kg. Dose increments were 5–10 fold in between cohorts for the first 5 cohorts and were then reduced to twofold increases. Two patients/cohorts were entered at the first 2 dose levels with increasing patient numbers beginning at the dose of 0.005 mg/kg (300 mcg: −50% RO). As of the cutoff date, doses of up to 2 mg/kg (120 mg) had been administered to patients with no DLTs or adverse events exceeding Grade 2, and no clinical symptoms or laboratory evidence of cytokine release. Based on the annual report of 2015, 6 patients had been enrolled at 4 mg/kg. The time from the IND submission to the dose of 2 mg/kg was approximately five years.

As human MTDs depend in part on the frequency of administration, dose frequency was examined for the antibodies. Of the 27 antibodies, 21 (78%) used every-2-week (Q2W) or less frequent dosing administration. Six of the 27 antibodies (22%) used weekly administration. The breakdown is presented in Table 6 below.

### 4. Discussion

With the growing number of immune activating products and nearly a decade of experience, we retrospectively examined the nonclinical data and some aspects of FIH clinical trial design. Among the questions to be addressed were the feasibility of using a common approach for FIH dose selection, the utility of animal toxicology studies in selection of the FIH dose and in clinical monitoring, and the length of time to complete Phase 1 trials given low FIH doses and traditional 3 + 3 dose-escalation design used for some products. In order to have a homogenous group of products,
we only included antibodies and CD3 bispecific antibody constructs. We later excluded CD3 bispecific products from FIH dose computation, due to low number of these constructs, structural heterogeneity, and schedule differences in administration. While some bispecific constructs are structurally comparable to antibodies with only minor differences (i.e. bivalent antigen binding), other products only minimally resemble antibodies, and the small size with corresponding shorter half-lives often led to daily administration in FIH trials. Daily administration can result in lower tolerance with reduced MTDs, requiring lower FIH doses compared to antibodies, which are mostly given Q2W/Q3W. In general, the incidence and toxicity grade of IRR/CRS was higher at low dose levels (e.g. in microgram ranges) for bispecific constructs compared to antibodies, further suggesting that CD3 bispecifics are not comparable to antibodies.

A variety of approaches were proposed by the sponsors for FIH dose selection, many of which were associated with receptor occupancy or activity. All approaches used by the sponsors resulted in FIH doses that were considered reasonably safe. As there is substantial variability in EC50s and hence in the calculated FIH doses, the question remains whether in the future there is a potential for a FIH dose that may be too high if it is based on activity data and activity studies are not optimized. We have only two INDs in our database with complete activity EC50s (one of which is a bispecific product for which the FIH dose was not analyzed) and thus cannot evaluate whether the range of FIH doses may encompass unsafe doses. We do not anticipate this to occur when assays are optimized. Activity EC50s generally had a wider range compared to dissociation constants, Kd. The range for Kd was up to 20 fold (highest-to-lowest), whereas the range of mean EC50s was up to 10,000 fold. Kd were obtained mostly through SPR, although other methods were also used. Dissociation constants had a tight range of up to 6 fold when Biacore or KinExA was used; this may be due to strict conditions of use provided by the manufacturer of these assays, indicating that there is an advantage in assay optimization, not just for activity assays but also for binding assays. Binding EC50s were obtained mainly by flow cytometry or ELISA and had a wider range compared to Kd; therefore, binding EC50 data were not used for estimation of FIH doses based on RO in this analysis as Kd values were available for all antibodies (except for one) and using Kd with a tighter range reduced the range of supportable FIH doses.

Activity assays are a good source of data to assess the potential for cytokine release induction in patients. Since the TGN1412 incident, emphasis has been given to CRA and conditions of the assay (FDA, 2014; Römer et al., 2011; Stebbings et al., 2007, 2013). Therefore, we examined CRAs conducted by the sponsors and conditions of use and compared the results (positive versus negative) to other activity assays. Of the 32 INDs, four did not contain an in vitro CRA either prior to FIH dosing or during the Phase 1 clinical trial. For approximately half the INDs, assays using both plate-bound and soluble platforms were conducted, and the other half used one platform only. Results of CRAs were used mostly for hazard identification, although it was used by some sponsors and OHOP reviewers for FIH dose selection as well. We could not identify any one activity assay that was the most sensitive or the most relevant in hazard identification among assays conducted as results varied depending on the product and the condition of use. The totality of data—to also include literature assessment and experience with other products targeting the same antigen—may be a better measure of hazard identification and could be used to decide whether a conservative approach for FIH dose selection may be warranted.

For the 27 antibodies examined, the FIH doses based on 20%–80% RO had acceptable toxicities (i.e. no drug-related death, no IRR/CRS greater than Grade 3, and overall manageable toxicities). There was no clear difference between immune checkpoint inhibitors and stimulators in terms of doses and occupancies that result in unacceptable toxicities. Doses above saturation also had acceptable toxicities for antibodies examined except for two Fc-modified antibodies with increased ADCC activity. A CD33 IgG1 with increased CD16A binding was associated with DLTs at a dose corresponding to slightly higher than 90% RO. CD16A is an activating receptor and increased CD16A binding has been linked to increased ADCC activity (Satch et al., 2006). A CD40 IgG1 antibody was afucosylated for increased ADCC activity and resulted in Grade 4 IRR/CRS in one patient at a dose corresponding to 90% RO. However, another IgG1 CD40 antibody had reduced CD16A binding and increased CD32B binding compared to the antibody with wild-type IgG1 Fc region and was administered to patients at doses above saturation with acceptable toxicities. CD32B is an inhibitory Fc receptor, although CD32B binding has been also linked to increased antitumor activity (Li and Ravetch, 2011; Stopforth et al., 2016). These results indicate that antibody engineering for modified effector function can affect the doses at which IRR/CRS events occur, with increased effector function potentially inducing IRR/CRS at lower doses compared to unmodified antibodies. Cytokine release as a function of Fc-mediated effector function has been previously described by multiple groups, such as Brennan et al. (2010) and Kirchies et al. (2012). Since we have a limited number of antibodies in our dataset with modifications to increase the effector function, further data collection on Fc-modified immune activating antibodies is warranted to examine the impact on patient safety.

For approximately half (12 out of 27 or 44%) of the antibodies examined the FIH doses were in microgram ranges and corresponded to up to 50% RO. These doses were 100s–1000s fold less than doses given to patients with acceptable/manageable toxicities and the period of time to complete the dose-finding trial was up to 5 yr (range of approximately 1–5 yr). While obtaining safety data is the main goal of Phase 1 trials, patients enrolling in clinical trials for cancer drugs have generally exhausted available therapies and enter with the hope of benefiting from the study. A clinical trial design that minimizes exposure to sub-therapeutic doses while maintaining safety is desired for these patients. This goal may be achieved by optimal FIH dose selection or through non-traditional FIH trial designs which permit intra-patient dose escalation when the FIH doses are low, such as ≤ 50% RO using Equation B. This latter approach has been used in a limited number of INDs, as in Example A, where the FIH dose was predicted to result in 10% RO and intrapatient dose escalation continued up to a dose corresponding to
approximately 70% RO. Another design to minimize the number of patients at low doses is provided in Example B, where single patients were entered at dose levels corresponding to predicted 6% and 50% RO and the design was changed to 3 + 3 at doses anticipated to cause receptor saturation. Example C describes a single dose administration of an immune checkpoint stimulator and is generally discouraged. Although all doses were well tolerated, many patients experienced disease progression and resulting death. A balance between safety and activity is desired in FIH trials; design of dose-finding trials should be discussed with the FDA review divisions during a pre-IND meeting.

When there were other antibodies in development or marketed targeting the same antigen, some sponsors conducted side-by-side binding and activity studies and proposed a FIH dose based on the comparative data. For these INDs, the FIH dose was generally near or above receptor saturation and the FIH clinical trial design was 3 + 3.

For many INDs, animal toxicity studies showed minimal drug-related effects, with findings predictable based on the pharmacology of the product. Toxicology studies were not used by the sponsors or FDA as the basis for FIH dose selection, only as supportive information to show that the FIH doses selected were well below doses given safely to animals. We assessed whether the traditional methods of FIH dose selection, 1/6th the HNSTD or 1/10th the NOAEL in animals (based on BW or BSA), could be used for FIH dose selection and concluded that these approaches are not safe because doses obtained may be above or too close to the doses that were safely administered to patients.

We questioned whether INDs that used low (e.g. microgram) doses of antibodies for initiation of a clinical trial were those submitted shortly after the TGN1412 event. There was no association. Several INDs with low FIH doses were submitted recently, e.g. 2013–2015.

In conclusion, for many immune activating antibodies, the FIH doses were low, requiring many dose escalation steps to reach an OBD, MTD, or a recommended dose for further clinical investigation. There is currently a need for optimization of FIH dose selection or optimization of FIH trial design to minimize the number of patients exposed to sub-therapeutic doses. Until a safe and yet efficient approach for FIH dose selection is established for these products, it may be best to optimize the FIH trial designs to allow rapid attainment of active therapeutic doses. For antibodies, receptor binding is directly associated with activity. For CD3 bispecifics, however, additional factors are involved such as binding to both targets, proximity of targets, and relative affinity towards the two targets—all of which can affect pharmacologic activity. Therefore, many factors should be considered for FIH dose selection for CD3 bispecific constructs and further data collection and appropriate modeling are warranted. Collaboration and data sharing among industry representatives may be needed for assessing and establishing more efficient approaches for FIH dose selection for immune activating products.

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Transparency document

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