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How Current Understanding of Clearance Mechanisms and Pharmacodynamics of Therapeutic Proteins can be Applied for Evaluation of their Drug-Drug Interaction Potential

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Abbreviations: TP, therapeutic protein; DDI, drug-drug interaction; P450, cytochrome P450; SM, small molecule; mAb, monoclonal antibody; IFNα, interferon α; IFNα-2b, interferon α-2b; IFNβ, interferon β; IL2, interleukin 2; IL-6, interleukin 6; hGH, human growth hormone; IL-1, interleukin 1; IL-10, interleukin 10; CRA, cytokine release assay; PK, pharmacokinetics; LBA, ligand binding assay

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Abstract

Increasing use of therapeutic proteins (TP) in poly-pharmacy settings calls for more in-depth understanding of the biological interactions that can lead to increased toxicity or loss of pharmacological effect. Factors like patient population, medications that are likely to be co-administered in that population, clearance mechanisms of a TP and concomitant drugs have to be taken into account in order to determine potential for drug-drug interactions (DDI). The most well documented TP DDI mechanism involves cytokine-mediated changes in drug metabolizing enzymes. Due to the limitations of the current pre-clinical models for addressing this type of DDI, clinical evaluation is currently the most reliable approach. Other DDI mechanisms need to be addressed on a case-by-case basis. These include altered clearance of TP resulting from the changes in the target protein levels by the concomitant medication, displacement of TP from binding proteins, modulation of Fcγ receptor expression, or others. The purpose of this paper is to introduce the approach used by Pfizer scientists for evaluation of the DDI potential of novel TP products during drug discovery and development.

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Introduction

There have been significant advancements in the development of Therapeutic Proteins (TP) over the past two decades. As a result, the number and diversity of biotherapeutic modalities on the market and at various stages of pre-clinical and clinical development have increased dramatically. As TPs are being more commonly used in poly-pharmacy settings, there has been an increase in the potential concern of TP drug-drug interactions (DDI) which may cause either a loss of pharmacological effect or an increase in toxicity. Some examples of clinically observed DDIs include 1.5-fold increase in trastuzumab serum levels by paclitaxel; 44% reduction of adalimumab clearance following multiple dosing of methotrexate; decreased exposure of simvastatin and omeprazole by 57% and 28%, respectively, following co-administration with tocilizumab. (Zhou and Davis, 2009). Early profiling for cytochrome P450 (P450) and transporter mediated DDIs, utilized pre-clinically to de-risk small molecule drugs, allowed safer and more successful therapies to be delivered to patients. However, TP DDI is a new and evolving scientific area with very few specific guidelines or consistent approaches available.

It is important that a systematic, science-driven approach should be used (Zhou and Davis, 2009; Lee et al, 2010; Huang et al, 2010). This approach includes understanding the pharmacology and clearance mechanisms of a TP, the patient population, and medications that are likely to be co-administered in that population, as well as potential mechanisms of DDI. Given the complexity of these factors and the limited knowledge in this area, currently clinical evaluation is the most reliable approach for studying TP DDIs.

The most well documented TP DDI mechanism involves cytokine-mediated changes in drug metabolizing enzymes. Multiple *in vitro* and a nu mber of *in vivo* human studies have demonstrated the effect of individual cytokines and their modulators on P450s and transporters (Prandota, 2005; Morgan et al, 2008; Strehlau et al, 2000; Morgan, 2009). In these cases, the TP plays the role of DDI perpetrator, while the victim is typically a small molecule (SM) drug.

Currently the most accepted pre-clinical approach for assessment of the effects of specific cytokine(s) and/or cytokine modulators on drug metabolizing enzymes and transporters utilizes human hepatocytes treated with physiologically relevant amounts of cytokines and/or cytokine modulators (Lee et al, 2010; Huang et al, 2010). This method allows for direct evaluation of the effect of T P on P450s and transporters, however, it has a number of li mitations and requires further optimization. Furthermore, translation of these *in vitro* findings to the *in vivo* setting has not been established at present. Potential in-silico approaches are being evaluated (e.g., Symcyp) however, these approaches suffer from the same lack of understanding of the basic mechanisms of TP DDI. Thus, it is likely that all cytokine modulators (cytokines, anti-cytokine mAbs, anti-cytokine receptor mAbs) will need additional scholarship on their DDI potential.

Since the hallmark of inflammation is the up- and down-regulation of cytokines, attenuation of pre-existing inflammatory states by cytokine modulators will likely alter systemic exposures of SM drugs towards exposures similar to those observed for populations in no n-inflammatory states and, therefore, it may not be necessary to address these types of DDIs early in development when studies are conducted using healthy volunteers. However, this will need to be evaluated in the appropriate patient population during drug development. Notwithstanding, there

may be situations when drug-metabolizing enzymes are expected to be modified in a manner which might result in an abnormal exposure of a SM drug. These situations present a safety concern, especially for SM drugs with narrow therapeutic windows that undergo therapeutic drug monitoring, and this type of interaction should be addressed during early development. This is not unique to TPs. Anti-inflammatory SM drugs can have similar effect on P450s and therefore, this mechanism of DDI should not be overlooked for SM drugs either.

DDI mechanisms, other than those mediated by cytokines, need to be addressed on a case-by-case basis. These include altered clearance of TP resulting from the changes in the target protein levels by the concomitant medication, displacement of TP from binding proteins (heparin effect on palifermin (Amgen Clinical Study Report: 20050137, 2007)), or modulation of Fcγ receptors expression which may result in decreased clearance of TP (methotrexate effect on adalimumab (Weisman et al, 2003; Bunescu et al, 2004)).

Another type of DDI can occur as a result of an immune response generated against one TP, which cross-reacts with a secondary administered TP. This type of DDI will not be discussed in this paper. Nonetheless, with increase in poly-therapy interactions via immunogenic response may become more common.

The purpose of this paper is to introduce the approach used by Pfizer scientists for evaluation of the DDI potential of novel TP products during drug discovery and development.

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Pre-clinical considerations for evaluation of cytokine-mediated DDI.

If a TP drug candidate is one of the cytokines for which the effect on P450s and transporters has been previously reported and characterized (IFNα, IFNα-2b, IFNβ, IL2, IL-6, hGH, IL-1, TNFα, or IL-10), or if it is a mAb directed against one of those cytokines or their receptors, no additional pre-clinical experiments are recommended. Given the inherent variability of *in vitro* systems, this class of BioTx would require a clinical DDI evaluation as described below.

If a TP drug candidate is a cytokine for which the effect on P450s and transporters has not been previously characterized, or if it is a mAb directed against that type of cytokine or its receptor, it is recommended that the effect of this TP on P450s and transporters be evaluated utilizing a human cultured hepatocyte assay. At a minimum, three different hepatocyte donors should be tested. In order to determine if this hepatocyte assay is appropriate, it is important to understand the mechanism via which the TP can modulate cytokines (i.e., direct versus indirect/down stream impact). The outcome from this assay can be used to qualitatively assess the likelihood of a DDI. Regardless of the results from current *in vitro* systems, clinical DDI evaluation may still be needed as discussed later.

Immunomodulatory TPs are typically evaluated for the potential to elicit cytokine release (TNFα, IL-6, INFγ, IL-8) in the *in vitro* human cytokine release assay (CRA) as part of a standard safety assessment. However, the current state of the art of this assay is limited to hazard identification using blood from healthy donors. Therefore, interpretations of cytokines generated in the CRA may not translate to *in vivo* situations, especially in diseased patients or in patients for whom the target does not exist in circulation. Since the effects on P450s for the four

cytokines listed above have been reported (Prandota, 2005; Morgan et al, 2008; Strehlau et al, 2000; Morgan, 2009), no additional experiments are recommended at this time.

All of these assays are qualitative in nature and should not be interpreted in a quantitative manner at this time. Further *in vitro – in vivo* characterization is ongoing to improve the future utility of these assays.

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Clinical considerations for evaluation of cytokine-mediated DDI

The interaction to be investigated in this case is the effect of a cytokine-modulating TP (perpetrator) on a SM drug (victim).

For TP DDI studies in which the mechanism of DDI is believed to be P450 mediated, it is recommended that clinical studies be conducted in patients rather than healthy volunteers, as P450 levels can be suppressed by inflammatory cytokines in patients. Anti-cytokine therapies can "normalize" P450 levels and, as a result, alter clearance of SM drugs. In healthy volunteers cytokine levels are not elevated, therefore cytokine modulators have little impact on P450 levels and would not be expected to alter P450 activity. Other important considerations for using patients instead of healthy volunteers include concerns surrounding the potential toxicity of either the TP or SM drug in healthy subjects, and pharmacokinetic (PK) differences for TP between patients and healthy subjects.

Individual substrate(s) for specific P450 enzymes should be considered for clinical evaluations which address the effects of TP on the PK of SM drugs. A less optimal, but acceptable, approach for assessing the effect of TP on the PK of a SM drug is the "cocktail approach" (Bjornsson et al, 2003). This type of evaluation can be conducted in Phase 1b/2/3 trials as appropriate.

For evaluation of the effect of TP on SM drugs, study designs including parallel-group, crossover (TPs with short half-lives), or single-sequence crossover designs (TPs with long half-life) (e.g., SM drug PK defined in patients during run-in period followed by SM drug PK defined following single and/or multiple dose TP administration) are commonly used. In addition, DDI assessments

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can utilize cross-study comparisons, which may involve comparison of data from healthy subjects versus patients. The results of such analyses should be evaluated as "hypothesis generating," and generally need to be confirmed.

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Pre-clinical and clinical considerations for evaluation of DDI mechanisms, other than those mediated by cytokines

Currently no pre-clinical studies are required for this type of DDI. DDI evaluation may be conducted on a case-by-case basis utilizing a risk-based strategy. The strategy should evaluate the probability of the TP drug candidate to be a DDI victim or perpetrator based on its clearance mechanisms and biological mechanism of action. These DDI studies can be conducted during the later stages of development. A population PK approach can be used for initial assessment, followed by a formal study if a DDI has been identified (Duan 2007, Huang et al. 2010). While a population PK approach can be used to evaluate TPs as both a perpetrator and a victim, blood samples collected during late phase studies are often utilized for determination of TP concentrations and thus, only allow for evaluation of TPs as a victim. To evaluate TPs as a perpetrator, concentration data for concomitant medications also need to be collected. It is important to remember that TPs can be involved in DDI with another TP, and not only with SM drugs. Underling mechanisms for these types of interaction could be more complicated and will require further investigation. Table 1 provides a list of questions that should help with gathering relevant information for determining if TP drug candidates are likely to be DDI victims and/or perpetrators. This template (Table 1) can be used as a tool for summarizing information for any type of DDI mechanism.

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Clinical considerations for TP intended for use in combination with other drugs (SM or another TP)

When a TP is designed to be co-administered with another drug (either SM or TP), *in vivo* DDI studies may need to be conducted even when there is no known mechanism of DDI. These types of studies should be considered in Phase 1/2 when the combination is first given to evaluate the feasibility of the combination and provide information for safety evaluation. Early evaluation of DDI with the combination drug can help set a development strategy and guide late stage study designs.

Interactions to be investigated include both the effect of the TP candidate on the concomitant drug (SM or another TP) and the effect of the concomitant drug on the TP candidate.

Selection of the interacting drugs for the DDI investigation is based primarily on the potential for concomitant usage, PK and PD properties of the compounds, and the therapeutic windows for selected drugs.

The effect of the TP on the concomitant drug is best evaluated using a within-patient comparison. Crossover designs can be applied for TPs with short half-lives, and parallel-group or single-sequence crossover designs (e.g., chemotherapy PK defined in patients during run-in period followed by chemotherapeutic PK defined following single and/or multiple dose administration of the TP) can be used for TPs with long half-lives. The possible effects of anti-

drug antibodies on the interpretation of crossover DDI studies of two BioTxs of the same modality will need to be considered.

The evaluation of the effect of a concomitant drug on the TP may be evaluated by cross-study comparison (e.g., TP PK defined in patients following TP treatment in combination with another drug (SM or TP), compared to TP PK as defined in a First-in-Patient single-agent TP study), especially when the TP has a long half-life.

Early evaluation of DDI between TP and SM used for cancer treatment is of particular importance because of the narrow therapeutic index of many anti-cancer drugs. While the consideration for general DDI evaluation of oncology TPs are similar to those of other therapeutic areas, challenges exist in conducting formal DDI studies in cancer patients. Unlike non-oncology TPs, DDI evaluations are not usually conducted as stand-alone studies, but rather a sub-study evaluation as part of an ongoing study. It is preferable to conduct DDI evaluations (when there is a sound rationale) during early Phase 1b/2 because this typically includes relatively small studies and operational issues can be better managed.

When DDIs are observed, appropriate labeling language will likely be required for the package inserts.

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Bioanalytical considerations

When *in vivo* DDI studies (either pre-clinical or clinical) are carried-out, potential interference of each concomitant drug on the quantification of the other should be investigated.

In most cases, TPs are quantified using different analytical platforms than SM drugs. Quantification of SM drugs typically utilizes liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) assays, while analytical methods for detection of TP are based primarily on ligand binding assays (LBA). The specificity and selectivity of LBA largely relies on the reagents used in the assays and the matrices in which the samples are collected. Since an extraction procedure is not always implemented during LBA analysis, the structurally related endogenous molecules, metabolic species, and other binding proteins or SM drugs could potentially interfere with the quantification of the TP of interest. It is therefore necessary to assess the interference of concomitant TP or SM drugs on the selectivity of the LBA used for quantifying the TP of interest during assay validation.

Conclusions

The study of TP DDIs is an evolving science, but clearly can have important clinical implications. It is crucial to interpret data carefully, in particular for cytokines as several cytokines are typically altered in parallel or sequentially. With that in mind, effort to understand the potential for DDIs should begin with an understanding of the pharmacology and clearance mechanisms of the TP and potential coadministered drugs, as well as the relevant patient population. While evaluation of TP DDI is still in its infancy, additional studies in this area will undoubtedly lead to improved preclinical tools to predict clinical outcomes and, ultimately, better guidelines for drug development.

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Participated in research design: Not applicable

Conducted experiments: Not applicable

Contributed new reagents or analytic tools: Not applicable

Performed data analysis: Not applicable

Wrote or contributed to the writing of the manuscript: Kraynov, Martin, Hurst, Fahmi, Dowty, Cronenberger, Loi, Kuang, Fields, Fountain, Awwad, Wang

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TABLE 1. Assessment of TP as potential DDI perpetrator or victim

Assessment of TP as DDI perpetrator	
1. TP is a cytokine, anti-cytokine, cytokine modulator, or c auses release of cytokines *in vivo* or in CRA	• Which cytokines are affected? • Have effects of those cytokines on P450 and transporters been previously reported? If Yes – no *in vitro* testing is required. If No – additional *in vitro* testing may be required to determine which P450s are likely to be affected • Is it known what cytokine levels are typical in the intended patient population?
2. TP candidate is intended to be used in combination with SM and/or TP drugs	• What is the therapeutic index of those SM or TP drugs? • What are the clearance mechanisms of the concomitant narrow therapeutic index (NTI) TP? • If NTI compound cleared via any of the P450s listed in 1, TP-DDI clinical investigation involving intensive PK profiling of NTI will probably be needed. If it is cleared via other pathways, PK data may be collected but not

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	necessarily via intensive sampling. • If any of the NTI clearance mechanisms (other than P450) are expected to be affected by the T P drug candidate, develop a risk-based strategy for addressing DDI
3. TP candidate is an immunomodulator	• Potential DDI if concomitant medication is a mAb and Fcγ receptors are involved in its clearance
Assessment of TP as DDI victim	
1. Therapeutic index of TP drug candidate	• If NTI, then develop a risk-based strategy for addressing DDI
2. Target and target type	• What are the biological consequences of inhibiting this target? • Soluble/cell surface target, and can it be shed? • Will blocking this target impact clearance of endogenous proteins etc?
3 Clearance mechanisms of TP drug candidate (target-mediated clearance, peptidates, binding proteins, Fcγ receptors, etc.)	• If contribution of target-mediated clearance significant at clinical dose and concomitant drug may impact target expression - potential for DDI
4. TP candidate (through its	• Potential for DDI

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mechanism of action) modulates expression of downstream receptors involved in elimination of concomitant TP or i ts own elimination.	
5. TP drug candidate interacts with endogenous proteins (other than target)	• Potential for DDI if concomitant medications alter levels of endogenous proteins that are involved in clearance of TP