の位置づけが確立していない。結果として、アジュバントについてはこれを構成する個々の成分単位で、それぞれを添加物として取り扱っている（平成22年度厚生労働科学研究費補助金「医薬品・医療機器レギュラトリーサイエンス総合研究事業－医薬品の品質、有効性及び安全性確保のための手法の国際的整合性を目指した調査と妥当性研究」平成22年度総括研究報告書、p.216「わが国の法的規制におけるアジュバントの位置づけについての考察」参照)。即ち、「新規アジュバント」の定義自体は上記の通りの解釈で間違いがないと考えられるが、このこととは無関係に、現状においてアジュバントは添加物としての位置づけに由来する拘束を受けている。したがって、現時点では複数の成分で構成されているアジュバントでは個々の成分それぞれについて「新規添加物」の定義に合致させた形で毒性評価を行う必要がある。言い換えればアジュバントの個々の成分について、③「例え使用前例があっても前例を上回る量が含まれて」いるのであれば、現状ではそれぞれに対して毒性評価を行う必要がある、ということになる。なお、「新規添加物」で必要とされる毒性として一般毒性（単回及び反復投与毒性)、遺伝毒性、生殖発生毒性、局所刺激性等が挙げられている。

アジュバントの毒性は、ワクチン製剤群とアジュバント単独群の比較によっても評価可能である。なお、アジュバントの投与量、投与頻度（回数）は、ワクチン抗原同様、予定臨床用量を基に設定すればよい。次回以降にも同じアジュバントが使用される場合であっても、使用量が増加する場合は「新規添加物」とみなされるため、新たなワクチン製剤の毒性試験でもアジュバント単独群を設けることが必要かもしれない。

なお、治療用ペプチドワクチンに用いられるアジュバントとしては、従来の脂質アジュバント以外にも、GM-CSF（顆粒級単球コロニー刺激因子）やIL-2（インターロイキン2）などの免疫賦活効果を有する医薬品が知られており、これらはワクチンとの同一製剤としてよりも同時投与で用いられる場合が多い。しかしながら、これらのいわゆるサイトカインアジュバントについては医薬品であることから、それを用いたワクチンは医薬品同士の併用という位置付けになるものと理解される。サイトカインアジュバントに対して必要な毒性試験については、使用量との関連性も含めてICH-S6 (R1) ガイドライン等を参照する必要がある。また、ワクチンの毒性評価に当たっては、他のアジュバントと同様、ペプチドとの相乗効果に留意する必要があると考えられる。

治療用ペプチドワクチンのための非臨床試験に関する コンシダレーションペーパー「注釈」

注 1. 図 1 解説

バイオ医薬品の場合には「適切な動物種」、即ち、その医薬品に対して薬理活性を有する動物種を選択することが前提となっている（図 1①）。これはバイオ医薬品の場合、on-target 毒性を中心に考えるものとされていることの現れである。

バイオ医薬品の中には「外来の標的（細菌、ウイルス）に対するモノクローナル抗体及び関連するバイオ医薬品」が存在する。ICH-S6 (R1) ガイドラインにおいては、こうしたバイオ医薬品の非臨床安全性試験について、病態モデル動物、即ち、宿主体内に医薬品の標的となる分子が感染した動物の使用を必ずしも一律に求めていない。これについては薬効試験の一環としてそのような病態モデル動物が用いられる場合に、そこに安全性評価を含めることも「可能」とするにとどめている。その理由は、病態モデル動物を用いた試験を安全性試験としてみなすにあたり、常にその妥当性の問題が伴うことにある。具体的には、病態モデル動物を用いた試験では背景データ等の集積が必ずしも十分でないため、得られた毒性変化の解釈が難しい、又は安全性評価のために必要とされる動物数の確保が容易でない等の問題である。このような種類のバイオ医薬品については、off-target 毒性の評価のために何らかの動物を用いた短期投与試験の実施を基本軸として掲げている。但し、その代わりに動物試験において on-target 毒性が評価できていないことに対応する形で、臨床においてそれ相応の十分なリスク管理措置を求めるという点が重要である（注 4 左図、赤字）。

感染症予防ワクチンの非臨床試験において「適切動物種」と称される場合、これは上記と同様の理由により病態モデル、即ちワクチンに対する免疫応答の標的となるべき外来抗原と同じ遺伝子・タンパク質を有している動物種を意味している訳ではない。ここで「適切動物種」とされるのは、外来抗原と同じ遺伝子・タンパク質の有無に関わらずワクチンに対して免疫応答を示す動物種、即ち「抗原非特異的な on-target 毒性」を評価できる動物種ということになる（図 1②）。

治療用ペプチドワクチンにおける「適切動物種」の考え方については、本文参照のこと。

注 2. 治療用ペプチドワクチンにおけるその他の非臨床安全性評価

図 2 以外の非臨床安全性評価としてはまず、「ヒト培養細胞を用いた *in vitro* 試験」が考えられる。これはサイトカインストーム等を含む予期せぬ免疫応答を引き起こす可能性が疑われる場合に実施が検討されて良い方法と考えられる。当該試験で検証する安全性懸念は、

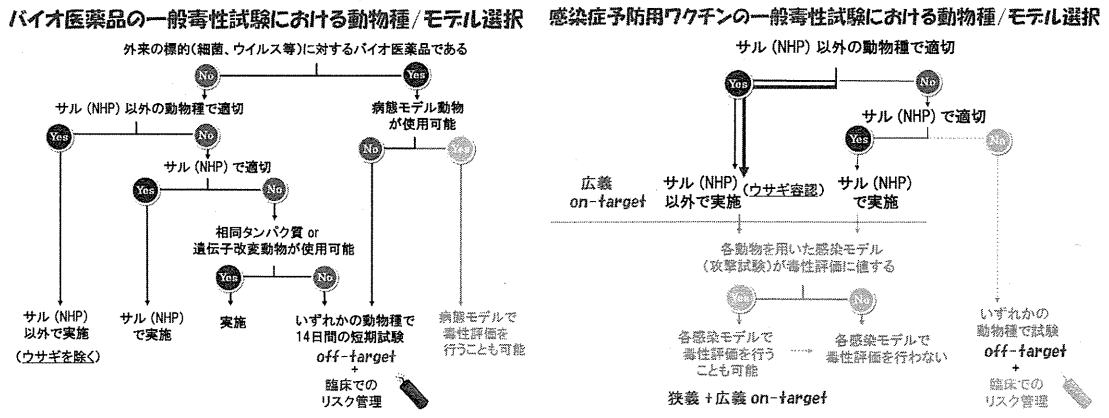
図 1③中の「抗原非特異的な on-target 毒性」に該当する。次に、「ペプチドの生理活性の有無を調べる *in vitro* 試験」、又は「ペプチドが生体内で目的外の受容体等に作用してその機能を競合阻害するか否かを検証する *in vitro* 試験」が挙げられる。これらの試験は図 2②の動物試験において該当する懸念が生じた場合等に実施が検討されるべきであろう。当該試験で検証する安全性懸念は、投与するペプチド自体が目的外の生理活性等を有するかどうかに関連するものであり、これは図 1③中の「off-target 毒性」に相当する。

注 3.　*In silico* データの利用及びヒト組織交差反応性試験の実施

細胞性免疫が免疫応答の主体となっている治療用ペプチドワクチンの場合、その由来が細胞質内において発現するタンパク質、分泌タンパク質又は細胞膜タンパク質のいずれであっても、当該ペプチドが HLA 分子により細胞上に提示されれば、T 細胞受容体によって認識されうる。したがって、*in silico* データの利用又はヒト組織交差反応性試験の実施に当たっては、正常細胞の標的抗原発現部位の相違によらず、発現の有無を検討しておくべきであろう。但し、ヒト組織交差反応性試験については、適用可能な抗体が入手もしくは製造困難となるケースも十分想定されることから、今後、代替となりうる方法について柔軟に考えておくべきである。加えて、HLA クラスⅠ又はⅡ分子の発現量、又は発現組織に対する特異的 CTL の細胞障害活性に関するデータ等の取得が可能である場合もある。また、ヒト正常組織における標的抗原の分布のみならず、抗原分子の機能が判明しているのであれば、関連する懸念の有無を判断する上で更に有用な情報となる。

注 4. バイオ医薬品及び感染症予防ワクチンの一般毒性試験における動物種/モデル選択

下図を参照。治療用ペプチドワクチンに関しては、本文図 3 に記載。



図の灰字部分は稀であるという意味。

注 5. CTL 誘導動物を用いた一般毒性試験の実施要否の判断（本文の図 3 灰字部分）

治療用ペプチドワクチンにおいて on-target 毒性の評価を行うために CTL 誘導動物を用いた一般毒性試験を行うことは、必ずしも良い方策とは言えない。しかしながら、下記の条件が揃えばその選択は必ずしも否定されるものではないと考えられる。

即ち、ある治療用ペプチドワクチンにおいて標的抗原が正常部位に発現することが明らかとなり、かつ開発者が添付文書において注意喚起レベルを下げる等、臨床でのリスク管理措置の軽減を望む場合には、この段階で CTL 誘導動物等を用いた on-target 毒性検討のための毒性試験を実施することが選択肢の一つとして浮上する。しかしながら、仮に CTL 誘導動物を用いて当該懸念を評価することを開発者が意図したとしても、実際に当該試験を実施する意義が生じるのは、「動物における免疫応答の内容に関しヒトとの十分な類似性が認められる場合」に限られると考えられる。具体的に言えばこれは、「マウスの正常細胞における標的抗原がヒトにおけるそれと十分類似した配列をもって疾患細胞上に発現した HLA 分子上に現れ」、かつ「マウスの CTL に発現したマウス自身の T 細胞受容体分子がヒトと十分類似した配列をもって標的抗原を認識する」と判断される場合である。CTL 誘導の方法として、マウスの相同ペプチドを投与する場合にも同様のことが言える。CTL 誘導の方法として HLA 導入マウス、相同ペプチドのいずれを用いるにせよ、当然のことながら治療用ペプチドワクチンの配列と動物における相当部分の配列の相同性が高いほど、免疫応答に関連する安全性評価に関し、動物からヒトへの高い外挿性が存在すると期待される。まとめて言えば、治療用ペプチドワクチンにおいて CTL 誘導動物を用いた試験により安全性評価を行うにあたっては、免疫応答の内容について動物とヒトの間に十分な類似性が認められることがその前提になると言える。

「狭義の on-target 毒性（疾患部位）」、即ち疾患部位に発現する標的抗原に対するワクチン免疫応答によりもたらされる毒性の評価の意義を否定するものではない。しかしながら、このような毒性評価は、担がん動物又は遺伝子改変による発がんモデル等の病態モデル動物を用いたメカニズム検討（MOA; mode-of-action）試験の中で、併せて安全性を評価することにより初めて「可能」とされる場合も多い。

CTL 誘導動物を用いた試験系の特殊性より考えて、これを動物種 2 種（げっ歯類及び非げっ歯類の 1 種ずつ）で行う必要性は低いと考えられる。現実には例え実施するにしても、マウス又はラットによるげっ歯類 1 種を用いた試験が妥当な選択となろう。

注 6. 治療用ペプチドワクチンにおいて「抗原非特異的な on-target 毒性」の評価を目的とした安全性評価を行う意義

治療用ペプチドワクチンにおけるもう一つの争点として、感染症予防ワクチンと同様に「抗原非特異的な on-target 毒性」のみの評価を目的とした安全性評価を行う意義があるか、という事項が挙げられる。結論からいうと、その意義は薄いと考えられる。理由は、感染症予防ワクチンにおいて「抗原非特異的な on-target 毒性」の評価が一律に求められている理由を考えれば逆に理解可能であろう。これは、感染症予防ワクチンが文字通り、主に「治療」ではなく「予防」に用いられているという使用状況と関係している。即ち、感染症予防ワクチンは大部分の場合、患者ではなくあくまで健康人に対して投与されるものであり、ベネフィットに見合うリスクを許容しにくいという問題が前提として存在する。また、実際に臨床において標的となる外来抗原と宿主における免疫が共存する時間は限られている。逆に言うと標的となる抗原が存在せず、ワクチンに対する免疫だけが誘導されている時間が被投与者個人の時間軸の中で大勢を占めているため、その分「抗原非特異的な on-target 毒性」について評価する意義が生ずる所以である。

それに対して、治療用ペプチドワクチンの場合はこの逆であり、i) 現時点では使用目的は「予防」よりも「治療」の要素が大きいため、ベネフィットに比べて比較的リスクを許容しやすく、更に臨床において宿主体内に標的となる抗原が存在する時間は比較的長期に亘る。もう一つの「抗原非特異的な on-target 毒性」に属する問題としては、ii) 治療用ペプチドワクチン投与に伴うサイトカインストームの懸念が挙げられる。実際、治療用ペプチドワクチンの長さや種類によっては、HLA class I 分子を通じて CTL（CD8 陽性細胞）を活性化するのみならず、HLA class II 分子を通じヘルパーT（CD4 陽性）細胞をも活性化する可能性のあることが報告されている（文献 1～4）。更に、CD4 及び CD8 陽性細胞の両方を活性化する複数の治療用ペプチドワクチンが混合されている場合には、サイトカインストームを引き起こす懸念が十分考えられる。当該懸念については、ヒト培養細胞を用いた *in vitro* 試験により適切な評価が行える可能性がある（注 2）。一方、CTL 誘導動物モデルを用いるサイトカインストームの予測は困難であるとされる。

以上、治療用ペプチドワクチンにおいては、ワクチン免疫応答の非特異的な作用に伴う毒性評価のみを目的とした動物試験を行う意義は乏しい。

注 7. 非臨床性安全試験での投与部位に関する「同一部位」の言葉の定義

げっ歯類の場合はサイズが小さいため、同側の上肢又は下肢ということで十分と考えられる。一方、ウサギを含めた非げっ歯類の場合はサイズが十分大きいため、同側の同一筋肉部位（例; 左三角筋）等とすべきであろう。参考までに、げっ歯類又はげっ歯類に関わらず、「同一部位」への投与を可能にするためには、投与毎に投与部位を油性マーキングペン（油性染料インキ）等でマークして次回の投与に備える方法等は有効と考えられる。

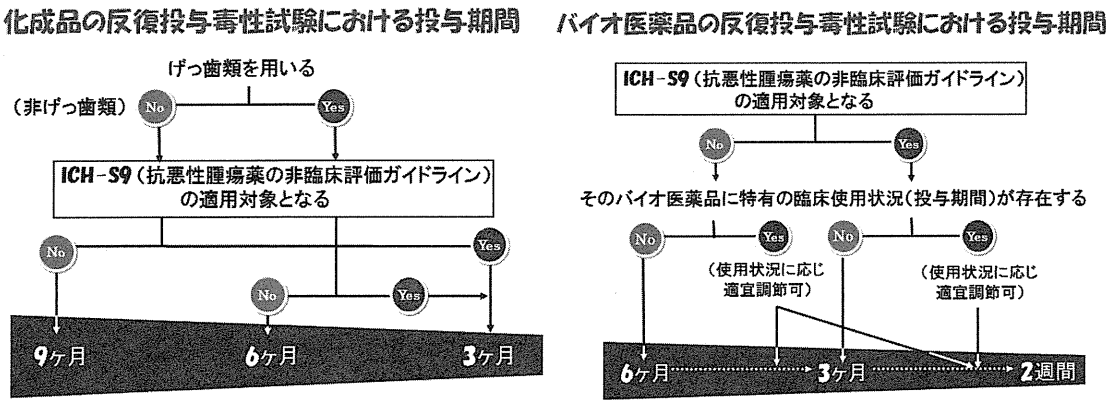
注 8. 非臨床における局所累積刺激性の評価 - 臨床試験実施前と承認申請時点での要求度の違い

非臨床における局所累積刺激性の評価については、臨床試験実施前と承認申請時点で、その要求度が異なるという点は考慮されて良いかも知れない。即ちまず、一般に臨床試験においてはエントリーされる対象者の条件と医師の元での十分なコントロールにより、「複数回の投与について可能な限り別部位への投与」を実現することは十分可能であろう。これに対し、製造販売後においては、例え添付文書において可能な限り別部位への投与を規定したとしても、患者の条件や医師によるコントロール条件に関する制約から、必ずしも別部位への投与を徹底できないという可能性があると考えられる。勿論、非臨床における局所累積刺激性評価は臨床試験前に完了していることが理想ではある。しかしながら、上に述べた理由により、局所累積刺激性の評価は承認申請時までに完了すれば良いとの考え方は許容可能と思われる。

注 9. 有効性の観点からのペプチドの薬物動態評価

治療用ペプチドワクチンにおいて、ペプチドの血中曝露量の測定は実施可能性及びその意義の両方の側面からみて不要とする旨の議論を展開したが、これは専ら安全性の観点からの結論である。これに対し有効性からの観点からは、薬物動態の評価がなされて良い場合があると言えるかも知れない。即ち、ペプチド未変化体の血中濃度が高く保持される場合や、動物に対してペプチドが大量に投与される場合等で、ペプチターゼによる分解が完全でない可能性もあると考えられる。そのような場合にはLC-MS等を用いてペプチドの曝露量を測定することが、有効性の観点からは有用かつ必要な情報となり得るかも知れない。

注 10. 化成品及びバイオ医薬品の反復投与毒性試験における投与期間　　下図を参照。



バイオ医薬品における「14 日間の off-target 試験」については、通常、反復投与毒性試験とは認識されて

おらず、実際、単回投与も許容可能と考えられていることから、右図には含めて考えていない（本文「局所毒性（局所刺激性）の評価について」参照）。

注 11. 図 5 解説

B（ペプチド治療用ワクチンにおける動物種/モデル選択について）の項で述べた通り、ペプチド治療用ワクチンにおいて実施される反復投与毒性試験は、「いずれかの動物種を用いた off-target 毒性評価のための試験」（図 3）が標準的な方法になると考えられる。当該試験において必要な投与期間は、げっ歯類、非げっ歯類のいずれを用いても、最低 2 週間、最高 6 ヶ月間程度が妥当と考えられるが、実際の投与期間は当該試験で評価することが可能な安全性懸念、そのうち特に局所累積刺激性の懸念の内容により適宜設定することが必要となろう。

局所累積刺激性の評価について、例えば予定される最高臨床投与回数を一週間間隔で 30 回としよう。このうち、どんなに投与部位（例えば皮下）を変えたとしても、5 回の投与分については、同一部位への投与が避けられないと予想されるものとする。この場合、局所累積刺激性を非臨床において評価するためには、皮下の同一部位への投与を一週間間隔で 5 回、望ましくはそれに加えて最低 1 回、即ち合計 6 回行えばよい。結果として、投与期間はこの場合、5 週間ということになる。また、一般に局所累積刺激性の非臨床における評価は、より回復期間の短い過酷な条件を選ぶという意味では、臨床での局所累積刺激性の投与間隔よりも短い間隔で実施することが可能であり、またそうすることが望ましい。その意味では、反復投与毒性試験が off-target 毒性評価のためのものとして実施される限り、上の場合において投与間隔を 1 週間ではなく例えば 5 日間として、投与期間を 25 日間（日日日週間）に短縮することは可能と考えられる。また、そもそもペプチド治療用ワクチンの種類によっては、局所累積刺激性の要素を極力ゼロに近いものとして制御可能な場合が存在するかも知れない。例えば、臨床における数十回の投与部位を何らかの方法により全て別部位に打ち分けることが可能である場合（但し、現段階では仮想的）や、治療用ワクチンであるにもかかわらず、最初から感染症予防ワクチン並に少ない投与回数を予定している場合等である。このような場合には、専ら局所累積刺激性を評価するための目的としてなされる off-target 毒性評価のための試験を、バイオ医薬品における off-target 毒性評価のための試験並に、14 日間に近づけることは可能かも知れない。

図 3 においては、可能性は低いものの CTL 誘導動物を用いた on-target 毒性評価のための試験を反復投与毒性試験とする選択も残されている。当該試験を行う場合の投与期間はバイオ医薬品の反復投与毒性試験の最高投与期間に準じてげっ歯類、非げっ歯類に関わらず 6 ヶ月間とすることが妥当と考えられる。但し、評価されるペプチド治療用ワクチンが専ら

進行がんに対する抗悪性腫瘍薬として用いられることが明らかならば、ICH-S9 ガイドラインを適用して、投与期間を 3 ヶ月間とすることが可能となろう。それに対し、当該ペプチド治療用ワクチンが進行がんの治療薬以外、例えばがんの早期治療薬や術後の維持治療薬等として用いられる可能性があるのであれば、ICH-S9 ガイドラインは適用すべきではないと判断される。

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ANNEX 1

World Health Organization

WHO consultation on the nonclinical and preclinical evaluation of adjuvanted vaccines

September 7-8, 2011
Rockville, Maryland, U.S.A.

Briefing page for presenters, moderators, and other participants on points for consideration

It is currently acknowledged that the nonclinical and preclinical evaluation of adjuvants and adjuvanted vaccines requires further consideration and refinement. Strategies and approaches for the development and delivery of vaccine antigens have expanded over the last decade. Antigens may require the presence of adjuvants for the induction of a potent immune response. Vaccines formulated with a range of adjuvants are currently in preclinical and clinical development and some have been licensed. However, the development and evaluation of some adjuvanted vaccines presents regulatory challenges as criteria to evaluate their safety profile may not exist, and toxicity study designs established for drugs may not be applicable to adjuvanted vaccines. Furthermore, manufacturers have questions about the type of information and extent of data that would be required to support proceeding to clinical studies with adjuvanted vaccines.

The purpose of this consultation is to summarize the scientific information, available data, outcomes of past scientific meetings on adjuvants, and identify critical questions that remain to be addressed from the perspective of regulated industry, toxicology experts, and regulators. Although WHO guidelines on nonclinical safety evaluation of vaccines exist (WHO TRS 927 Annex 1, hard copy provided on site),they contain very brief special considerations for adjuvants and adjuvanted vaccines.

This consultation will initiate the process of drafting a WHO guidance document for the nonclinical and preclinical evaluation of adjuvanted vaccines. Clear guidance on this topic should allow manufacturers and regulators to proceed in an efficient manner on the critical path towards development and licensure of adjuvanted vaccines indicated for the prevention of diseases with important global public health impact, including but not limited to, HIV, malaria, and pandemic influenza.

The meeting objectives are:

- To host a discussion on regulatory considerations for nonclinical and preclinical evaluation of adjuvants and adjuvanted vaccines;
- To determine the specific points for consideration on the nonclinical and preclinical evaluation of adjuvanted vaccines that need to be addressed in a guidance document geared to manufacturers and regulators; and,
- To develop an outline for WHO written guidance on nonclinical and preclinical evaluation of adjuvanted vaccines.

World Health Organization

The expected outcome of this initial consultation is an outline of a written WHO guideline document that would address specific points to be considered for nonclinical and preclinical evaluation of adjuvanted vaccines geared towards regulatory registration. The outcome of the consultation will be reported to the WHO Expert Committee on Biological Standardization (ECBS) for consideration in October 2011.

Potential discussion points to consider in the proposed WHO document on nonclinical and preclinical evaluation of adjuvanted vaccines include:

Manufacturing information

- Assessment of vaccine potency in complex formulations
- Adjuvant/antigen interactions and stability
- Assessment of adjuvant "activity"

Evaluation of stand alone adjuvants (e.g. mixed with vaccine antigens prior to administration) vs. adjuvanted vaccines that are engineered to contain the adjuvant component(s) as part of the final product

Multi-component adjuvants and what constitutes a "significant change"

- When would a change in one or more components (or excipients) require new nonclinical evaluation of the modified adjuvant?

Incorporation of human tissue culture-based assays in the nonclinical evaluation of adjuvanted vaccines, in particular when animal species barrier exists (e.g. cytokine responses, cell marker expression, others)

Animal models to use in nonclinical studies and applicability to humans

- Evaluation of proof of concept regarding mechanisms of action (e.g. induction of cellular immune responses)

Early clinical trials

- Questions to be considered/addressed to meet regulatory expectations prior to initiating a Phase 1 study

All participants are encouraged to share their experiences with testing and characterization of their adjuvants and adjuvanted vaccine, discuss the challenges faced before initiating clinical trials, and the lessons learned from failures and successes in the clinical studies. Presenters may want to consider describing their experiences through case studies, highlighting areas where regulatory guidance on nonclinical or preclinical evaluation of adjuvanted vaccines may have been helpful. The ultimate goal of the WHO document is to provide guidance on important regulatory considerations and possible requirements for nonclinical and preclinical evaluation of candidate adjuvanted vaccines to support use in clinical trials, and ultimately for licensure/marketing of the product. In-country NRAs would have the choice to decide their own regulatory requirements or adopt WHO guidance.

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Appraisal of state-of-the-art

Overview of global regulatory toxicology requirements for vaccines and adjuvants

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ABSTRACT

This paper provides an overview of the legislations and regulatory approaches currently applied to the nonclinical safety assessment of human preventive vaccine products in three ICH regions, i.e., the EU, USA, and Japan. Perspectives of the three regions with regard to the various types of toxicity studies currently considered to assess the nonclinical safety of preventive vaccines are compared and described in more detail than in published guidelines. In addition, the common issues and current challenges in nonclinical safety assessment of preventive vaccines are discussed.

1. Introduction

Non-clinical safety evaluation plays an essential part in the overall development of vaccines. During the nonclinical and clinical development stage, strategic planning of the toxicity testing program for a given vaccine product is a key for developers and producers to achieve clinical trial or marketing approval in a timely manner.

Most commonly, vaccine developers and producers design their specific toxicity testing programs by consulting guidance documents, that have been released in the past from the EMA (EMA, 2010; EMEA, 1997, 2001, 2002, 2005, 2007, 2008a, 2008b), the FDA-CBER (www.fda.gov, 2006, 2007, 2010), ICH (ICH Topic, 1998), or the WHO (www.who.int, 2003; Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines, 2007) (Table 1). However, existing guidelines are general in nature and frequently, lacking sufficient detail for recommendations on specific testing programs. In addition, it has already been recognized that the regulations and regulatory requirements of different countries or regions can differ, at least to some extent. For these reasons, many vaccine developers and producers have interest in a better understanding of the current global regulatory environment and thus meeting regulatory expectations necessary for marketing approvals worldwide. It was in this context that in July 2010 a continuous education course-2 (CEC-2) was organized during the XII international Congress of Toxicology held in Barcelona, to discuss the actual perspectives from the EMA, FDA-CBER, WHO and Japan.

2. Purpose of the workshop

The purpose of the CEC-2 workshop was to provide an overview on regulatory toxicology and risk assessment processes for vaccine development. In Workshop Session 1, the global regulatory environment, i.e., the perspectives of EMA, FDA-CBER, and Japan were discussed by Dr. Yuansheng Sun (PEI, Germany), Dr. Marion Gruber (US FDA, CBER, USA), and Dr. Mineo Matsumoto (Office of Biologics, PMDA, Japan), respectively. These perspectives focused mainly on preventative vaccines. In addition, common issues and current regulatory challenges related to nonclinical toxicity testing were discussed by Dr. Gruber. In this paper, the authors summarize these perspectives and discussions in Sections A–C.

3. Section A: general remarks of EU, US and Japan

3.1. EU

3.1.1. EU legislation and regulatory network

Under the EU regulatory network, vaccines are classified as pharmaceutical products (as first defined by the amended Council Directive 65/65/EEC, ref. Council Directive, 1965) and have since 1993 been regulated as special pharmaceuticals under the legislative

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Table 1
Guidelines for the nonclinical safety assessment of vaccines.

Regulatory agency	Vaccine type	Guideline
World Health Organization (WHO)	All vaccines	WHO guidelines on nonclinical evaluation of vaccines (www.who.int, 2003)
	DNA vaccine	Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines (Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines, 2007)
International Conference on Harmonization (ICH)	Recombinant vaccines	ICH S6 and ICH S6 (R1): Preclinical safety evaluation of biotechnology-derived pharmaceuticals (ICH Topic, 1998)
European Medicines Agency (EMA)	All vaccines	Note for guidance on Preclinical pharmacological and toxicological testing of vaccines (EMEA, 1997)
	Adjuvants for human vaccines	Guideline on adjuvants in vaccines for human use (EMEA, 2005)
	DNA and vector-based vaccines	Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal products (EMEA, 2001)
		Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA, 2008a)
		Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines (EMA, 2010)
	Smallpox vaccines	Note for guidance on the development of vaccinia virus-based vaccines against smallpox (EMEA, 2002)
	(Pre)Pandemic influenza vaccines	Guideline on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context (EMEA, 2007)
		Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (EMEA, 2008b)
United States Food and Drug Administration (FDA-CBER)	All vaccines	Guidance for Industry: " Consideration for developmental toxicity studies for preventive and therapeutic vaccines for infectious disease indications" (www.fda.gov, 2006)
	DNA vaccines	Guidance for Industry:"Considerations for Plasmid DNA Vaccines for Infectious Disease Indications" (www.fda.gov, 2007)
	Viral vaccines	Guidance for Industry: "Characterization and qualification of cell substrates and other biological materials used in the production of viral vaccines for infectious disease indications" (www.fda.gov, 2010)

instrument (Council Directive 89/342, ref. Council Directive, 1989), to fulfil all necessary requirements for human pharmaceutical products (as laid down in Directive 75/318, ref. Council Directive, 1975). Of note, these Directives have nowadays been replaced by the recent Directive 2001/83/EC, as amended (Directive, 2001). Thus, vaccines must undergo nonclinical safety evaluation before clinical investigation can be initiated, in order to support clinical trial or marketing approval. These EU regulatory requirements are described in Council Directives related to conduct of clinical trials and marketing authorization.

In Article 3, chapter 2 of the Directive 2005/28/EC (Commission Directive, 2005), it is stated that "the available nonclinical and clinical information on an investigational medicinal product shall be adequate to support the proposed clinical trial".

In 4.2 content: basic principles and requirements, Module 4 of the Directive 2001/83/EC (Directive, 2001), it is stated that:

1. " the pharmacological and toxicological tests must show
   a. the potential toxicity of the product and any dangerous or undesirable toxic effects that may occur under the proposed conditions of use in human beings; these should be evaluated in relation to the pathological condition concerned;
   b. ...all results must be reliable, of general applicability..."
2. "for biological medicinal products such as vaccines... the requirements of this Module may have to be modified...the testing program carried out shall be justified..."

The European legislative documents including the Council Directives and Regulations as well as European Pharmacopoeia consistently link the term "vaccine" exclusively to those products for prevention or treatment of infectious diseases. Collectively, the vaccine is defined as "*agents that contain antigens(s), produce active immunity, with the aim at preventing or treating infectious diseases*". Therefore, criteria for classifying a medicinal product as a vaccine product should consider the relation of antigen(s) to infecting agent(s), irrespective of the forms of antigen, as well as the intended indication to prevent or treat infectious diseases.

Especially, it is of note that vaccine products against infectious diseases fall outside of the legal definition of advanced therapy medicinal products (ATMPs) including gene therapy medicinal products (GTMPs) and somatic cell therapy medicinal products (sCTMPs), as clearly described in recent new Annex I to Directive 2001/83/EC (COMMISSION DIRECTIVE, 2003).

According to the definition, vaccines will therefore be a highly diverse class of medicinal products that, in some instance, may be overlapped with medicines of the ATMP category. Vaccine products regulated by the EU include (but not restricted to) whole microorganisms, inactivated or live attenuated; chimeric microorganisms, virus-like particles, antigens extracted or purified from microorganisms, or secreted by them, synthetic antigens (e.g., peptides), antigens produced by recombinant DNA technology, live recombinant vectors, or plasmid DNA.

There is a highly-interactive network for vaccine regulation in the EU. For clinical trial applications, authorization of clinical trials remains the responsibility of national authorities of Member States where the trial is conducted. As a European Union body the EMA (in London) is hosting the clinical trial coordination group, to discuss common principles and processes to be applied through the European medicines regulatory network. The EMA is mainly responsible for coordinating the existing scientific resources at its disposal by Member States for evaluation, supervision and pharmacovigilance of medicinal products. This regulatory body also contributes to publishing vaccine Guidance documents by different working parties and expert groups such as Safety Working Party (SWP), Gene Therapy Working Party (GTWP), Vaccine Expert Group (VEG) subsequently becoming Vaccine Working Party (VWP), and Biologicals Working Party (BWP). For marketing authorization of some vaccine products, such as recombinant vaccines, vaccines for treatment of AIDS, and those belonging to the GTMP category, a centralized procedure is mandatory (Regulation (EC), 2004). As a European Union body the Sanco is responsible for laying down the Directives, Legislations and Regulations.

3.1.2. EU current approach/applicable guidelines

The EMA published several guidance documents that address the nonclinical toxicity evaluation of vaccine products (Table 1). The *Note for Guidance on Preclinical Pharmacological and Toxicological testing of Vaccines (CPMP/SWP/465/95)* (EMEA, 1997) and the *Guideline on Adjuvants in Vaccines for Human use (EMEA/CHMP/VEG/134716/2004)* (EMEA, 2005) are the two regulatory documents recognized

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as most comprehensive and formally applicable to a variety of vaccine products regulated in the EU. However, for live recombinant viral vectored vaccines and for those considered to be in the GTMP category (e.g. plasmid DNA) or biotechnology-derived pharmaceutical category (e.g. antiidiotypic vaccines, monoclonal antibodies as immunogens), the specific guidelines are of use (EMA, 2010; EMEA, 2001, 2008a).

In this workshop, the EU perspective was mainly discussed based on the following two comprehensive European Guidelines. Other disease-orientated EMA/CHMP Guidelines were only briefly discussed.

3.1.2.1. CHMP guideline on adjuvants in vaccines for human use. The term "adjuvant" in this Guidance is defined as *a component that increases specific immune responses to an antigen.* It was subsequently clarified, in an explanatory note adopted in 2006 (EMEA, 2006), that an adjuvant should be part of the (reconstituted) vaccine formulation that is administered simultaneously or concomitantly with the vaccine antigen. Thus, an adjuvant is only licensed as part of the final product. Compounds that are given separately and/or at a different time point are not considered to be adjuvants but are called immunomodulators.

The Guidance is relevant for quality, nonclinical and clinical matters pertaining to all preventative vaccines containing an adjuvant and also applicable to quality and nonclinical aspects of adjuvanted therapeutic vaccines.

For acceptance of an adjuvant present in a vaccine, the benefits must be weighed against the risk of any potential adverse reaction inherent to it. A large emphasis is put on safety for preventative vaccines because they are given to a predominantly healthy and, in most cases, pediatric population. The picture is different for therapeutic vaccines that are usually administered to seriously ill patients or high-risk groups, thus, the benefit of vaccination can be substantial and, therefore, an increased level of toxicity may be acceptable.

The Guidance is applicable to both new and established adjuvants, though for the latter, necessity for compliance will vary on a case-by-case basis. In the EU, several new adjuvants have recently been licensed as components of vaccine formulations for the human use, including MF59 (component of influenza vaccines Fluad® and Focetria®), AS03 (a component of influenza vaccine Pandemrix®), AF03 (a component of influenza vaccine HUMENZA®), AS04 (a component of hepatitis B vaccine Fendrix® and human papillomavirus vaccine Cervarix®).

The introduction of an adjuvant into a vaccine presents regulatory challenges. The lack of universality of mode of action, the complexity of immune mechanisms involving multiple factors (cells and cytokines/chemokines), as well as the complex nature of adjuvant–antigen interactions preclude safety data extrapolation from one adjuvant–antigen combination to another combination, and represent key regulatory concerns. Hence, the individual adjuvant–antigen combination and route of vaccination that is licensed may need to be evaluated on a case-by-case basis. The extent of nonclinical toxicity data expected would rest upon the level of information available for the selected adjuvant. See below for more detail about this guideline (Section B–8.Adjuvants).

3.2. US

3.2.1. US legislation and regulatory considerations

Preventive vaccines for infectious diseases indications are biological products that are regulated by the Office of Vaccines Research and Review (OVRR) in the Center for Biologic Evaluation and Research (CBER) of the Food and Drug Administration (FDA). Vaccines undergo a rigorous review of laboratory and clinical data to ensure their safety, efficacy, purity and potency prior to marketing. The legal framework for the regulation of vaccines derives primarily from Section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262) and from certain sections of the Federal Food, Drug and Cosmetic Act (FFD & C Act) (505b) (www.fda.gov).

Vaccines are a heterogeneous class of medicinal products containing antigenic substances capable of inducing specific, active and protective host immunity against an infectious agent or related product. In contrast to other biological products that are predominantly developed to treat ill patients, vaccines are given primarily to large numbers of healthy people, predominantly healthy infants and children, and this places significant emphasis on their safety. With the development of a broad range of novel vaccines, the composition of vaccine products has evolved from attenuated or inactivated whole cell organisms to protein–polysaccharide conjugates, peptides, recombinant proteins, DNA vaccines, and gene transfer products. These products are often combined with novel adjuvants, administered in new delivery systems, and administered by new routes of administration. The determination of the nonclinical safety of the product includes the development of a manufacturing processes that results in consistent product manufacture and characterization, as well as preclinical safety studies in animal models that are a frequent prerequisite to move a vaccine from the laboratory to the clinic.

Safety concerns regarding investigational preventive vaccines include potential adverse effects due to inherent toxicities of the product, toxicities of impurities and contaminants, toxicities due to interactions of the vaccine components in the vaccine formulation as well as toxicities linked to the immune response induced by the vaccine.

3.2.2. Applicable regulations

In the US, before a clinical investigation of a vaccine can be initiated, a sponsor must submit to the FDA an Investigational New Drug Application (IND) (Novak et al., 1997). The information that must be submitted in support of an IND is described in the US Code of Federal Regulations (CFR), Title 21 CFR 312 (www.accessdata.fda.gov, 2010). The regulations in Title 21 CFR 312.23 (a) (8) specify that adequate information about pharmacological and toxicological studies either *in vivo* or *in vitro* should be presented on the basis of which it can be concluded that it is reasonably safe to conduct a proposed clinical investigation. The regulations also states that the kind, duration and scope of animal and other tests required will vary with the duration and nature of the clinical investigations. The CFR specifies that each nonclinical laboratory study should be conducted in compliance with GLP (21 CFR part 58), or if the study was not conducted in compliance with those regulations, a brief statement of the reasons for the non-compliance should be provided.

Title 21 of the CFR, Section 600.3 defines safety as "the relative freedom from harmful effect to persons affected directly or indirectly by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time." Due to the diversity of preventive vaccine products, applying these criteria requires careful consideration of the character of the product, the methods of manufacture, the clinical indication and circumstance under which the product may be administered. Thus, information on the intended target population, the proposed route of administration, available clinical data from the use of related products, available information on the mechanism of action of the product, and features of the product, especially novelty is needed to determine whether a toxicity study in animal models is needed. Another important consideration is whether animal models exist that can provide meaningful safety information that would allow proceeding to Phase 1 clinical trials.

Toxicology studies in animal models can provide data to support the conclusion that it is reasonably safe to proceed to a clinical investigation. Thus, before proceeding to clinical trials, for preventive vaccines, it is important to establish a safe and immunogenic dose in animals, to identify potential target organs for toxicity, and to identify safety parameters for clinical monitoring. While it is recognized that currently available animal models are limited in their ability to detect

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rare toxicities or specific toxicities that may occur in a particular human subpopulation, toxicology studies using currently available animal models are nevertheless a critical tool to assess the preclinical safety of the product. Nonclinical safety studies are usually conducted or proposed when the investigational product contains a novel vaccine antigen, DNA vaccines, vaccines formulated with an adjuvant or administered by a novel route of administration (RoA). For these products the type of studies performed typically include repeat-dose toxicity studies, biodistribution studies, when applicable (e.g., DNA vaccines), and reproduction toxicity studies (e.g., for products indicated for a population that includes females of childbearing potential). With the advent of vaccine antigens that are combined with novel adjuvant to increase the immune response to the vaccine antigen the US FDA is currently re-evaluating its approach to nonclinical safety assessment of vaccines, in particular its applicability to testing the adjuvant component.

3.2.3. US FDA current approach/applicable guidelines

The US FDA approach to nonclinical safety testing of preventive vaccines is summarized in the guidance document entitled "WHO guidelines on nonclinical evaluation of Vaccines," published by the WHO in 2003 www.who.int/biologicals/publications/nonclinical_evaluation_vaccines_nov_2003.pdf (www.who.int, 2003). This document provides basic principles and approaches to nonclinical safety evaluation of vaccines that are based on a case-by-case approach and allow flexibility for testing requirements. The document represents an effort to globalize and harmonize recommendations and requirements for nonclinical safety evaluation of preventive vaccines across regulatory agencies by outlining the international regulatory expectations during the various development phases of these products. The document presents a position on nonclinical evaluation of vaccines that is recognized by the US FDA. In addition to the WHO guidance, several US guidance documents were developed to specifically address the nonclinical safety of preventive vaccine products (www.fda.gov, 2006, 2007, 2010).

3.3. Japan

3.3.1. Japanese regulatory considerations for vaccines

In light of the approval records in Japan thus far, the word 'vaccine' in this country automatically connotes vaccines aimed at preventing infectious diseases. Therapeutic vaccines, such as those against cancers, have not been approved in Japan to date, although they are currently being developed by industry and/or academia. This session focuses on traditional, preventive vaccine types, and introduces a new regulatory guideline on vaccines developed in Japan (Guideline for Nonclinical Studies of Preventive Vaccines for Infectious Diseases, 2010).

There has been a serious gap in recent approval numbers between Japan and other countries; which is referred to as the 'vaccine gap'. For instance, the number of vaccines approved in Japan from 1984 to 2009 is slightly more than half of the number in either the EU or USA. It has been suggested that one factor in this gap is whether or not respective regulatory divisions have specific guidelines for vaccines. For example, in contrast to Japan, the EU published a guideline for nonclinical studies for vaccines in 1997 (EMEA, 1997) and the US FDA published a guideline in 2006, although the FDA guidance is confined to the issues of reproductive and development toxicity studies (www.fda.gov, 2006). The WHO guideline for nonclinical studies of vaccines (www.who.int, 2003) could influence Japanese regulatory approaches to nonclinical safety assessments of vaccines, however, the Japanese people wanted Japan's guidelines to directly define what kinds of nonclinical data are required for vaccine development.

Such societal demand indeed prompted two serial government-funded research projects. The first project, led by Dr. T Inoue, of the National Institute of Health and Sciences (NIHS), was conducted from 2004 to 2007 and was entitled 'Studies on the methodologies of nonclinical studies for particular pharmaceuticals such as vaccines and anti-cancer drugs'. Based on the research outcomes of this project, another project, led by Dr. K. Yamanishi, of the National Institute of Biomedical Innovation (NIBIO), was conducted from 2007 to 2010 and was entitled 'Studies on making guidelines for vaccine developments'. This project covered both clinical and nonclinical issues. The nonclinical as well as clinical guidelines were finally released on May 27, 2010, as results of Dr. Yamanishi's work. The former was named 'Guideline for Non-clinical Studies of Preventive Vaccines for Infectious Diseases' (Guideline for Nonclinical Studies of Preventive Vaccines for Infectious Diseases, 2010). A large portion of this manuscript from here will deal with what is written in this guideline (Guideline for Nonclinical Studies of Preventive Vaccines for Infectious Diseases, 2010). The English translation can be obtained as Supplementary data.

The scope of the guideline is preventive vaccines against infectious diseases, but not therapeutic vaccines against non-infectious diseases, such as the so-called 'cancer vaccines'. As for the vaccine formulae, the guideline states that it covers live attenuated vaccines, inactivated vaccines, toxoidal vaccines, and recombinant protein vaccines, but does not cover passive immunization with antibodies, anti-idiotypic antibodies, peptide vaccines, or DNA vaccines and 'cell vaccines'. This definition of the scope is considered virtually the same as that of the WHO guideline (www.who.int, 2003). On the other hand, it seems to be somewhat different from the FDA guidance, whose scope includes DNA vaccines but not recombinant protein vaccines (www.fda.gov, 2006).

4. Section B: the detailed subjects in the guidelines of EU, US and Japan

4.1. General issues

4.1.1. Basic principals

The scope of the EU's guidance covers only *new* vaccines (EMEA, 1997). The definition of the terms "*new*" and "*types*" of vaccine products to which this guidance formally applies are explicitly specified in the guideline. The potential safety concerns that may be related to vaccine products, such as general systemic toxicity, induction of local toxicity, pyrogenicity, paradoxical enhancement of intended diseases, autoimmunity and sensitization, and teratogenicity, were outlined. With these potential concerns in mind, all the items or testing areas that should be carefully addressed were listed. However, the Guidance allowed some flexibility in the selection of investigation. Although the contents of the guidelines differ to some extent from those stated in the WHO guideline and the Japanese guideline, all guidelines consistently recommend a 'case-by-case Approach' in nonclinical safety assessments of vaccines.

4.1.2. Species selection

Since potential inter-species differences in immune systems exist and immune-mediated reactions may constitute a source of potential toxic effects of vaccine products, the relevance of species selection in toxicity studies is critical and should be carefully evaluated. Ideally, animal models should provide an intended spectrum (cellular, humoral) and comparable types of responses (to humans) and be susceptible to pathogenic organism infection. At a minimum, the animals should be able to develop an immune response to the vaccine antigen and a relevant animal is currently defined as a species capable of mounting an immune response to the vaccine antigen. There is no discrepancy in the policies among the EU, the US and Japan.

One relevant species is in general, sufficient to conduct toxicity studies for preventive vaccine products. This differs from that of 2-species recommendation for biotechnology-derived products (ICH-S6). This 2-species approach also applies to new adjuvant for

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human vaccines as stated in the EU adjuvants' guideline (EMEA, 2005) (see below: *The detail of CHMP Guideline on Adjuvants*).

4.2. The single-dose toxicity study

In the EU, stand-alone toxicity studies are not generally requested. The EU guidance (EMEA, 1997) indicates this type of study can be integrated into an immunogenicity or safety pharmacology study, or be replaced by a repeat-dose toxicity study. Of note, the study parameter "clinical pathology" is not mentioned in this guidance. This parameter should be included as indicated in the WHO guideline (www.who.int, 2003), both for stand-alone and for combination toxicity/immunogenicity studies.

This concept does not differ among the three regions, e.g., US, EU and Japan. In Japan, the term 'acute toxicity study' instead of 'single-dose toxicity study' is used and coincides with the ICH-M3(R2) guideline which takes into account the 3R (reduce/refine/replace) principle.

4.3. Repeat-dose toxicity study

This study design is considered pivotal to evaluate multiple-doses administration of vaccine formulation suggested for immunizations of humans. The study design should include appropriate control groups, i.e., to compare the reactogenicity of the investigational vaccine to a placebo, to evaluate reversibility of potential observed adverse events and to screen for potential delayed adverse effects.

The dose administered to animals depends on the planned clinical dose and the expected immune response induced by the vaccine. To better simulate the proposed clinical usage, vaccine doses should be given as episodic doses, rather than daily doses. If feasible, one full human dose (mL or mg/body weight) should be administered, not scaled for body weight or surface area. When it is not feasible to administer the full human dose, a dose that exceeds the human dose on a mg/kg bases and that induces an immune response in the animal model may be used. Alternatively, it may be possible to administer the total volume to more than one site using the same route of administration. The route of administration should correspond to the intended clinical route of administration. In general, at least one additional dose, relative to the clinical trial, should be incorporated into the study design ($N+1$). This recommendation is consistent among the 3 regions, i.e., US, EU and Japan. In Japan, frequency of dosing in animals can be equal to frequency of dosing in human in those situations where the vaccine's mechanism of action is already well known, or where sufficient dose is administered in light of the body weight conversion (e.g., >10) relative to the clinical trials.

To simulate the proposed clinical dosing regimen, vaccines should be administered as episodic, rather than daily, doses, whereby the dosing interval may be reduced (e.g., 2–3 week interval). This principle is stated in the WHO guidance (www.who.int, 2003). The EU region generally accepts the proposed episodic dosing using 2–3-week intervals, however in certain situations, e.g., where kinetics of the antibody responses (antibody levels or other intended immune responses) is poorly known, studies may be needed to evaluate the primary and secondary immune responses over an extended period of time in order to justify the minimal interval in study design.

All three regions, i.e. US, EU and Japan encourages incorporating an evaluation of immunogenicity parameters in addition to an assessment of toxicity parameters into the design of the study. The rationale for this is to support the choice of the animal model and to reduce the animal use. The WHO guidance recommends that a broad spectrum of information be obtained such as the potential for local inflammatory reactions, systemic toxicity, and effects on the immune system. In-life parameters to be monitored should include clinical observations, such as general health, body temperature, weekly body weights and weekly feed consumption. Interim analysis of haematology and serum chemistries should be conducted following episodic dose administrations, i.e., usually at pre-test as well as after first and last test article administration. Local toxicity should be evaluated using a grading system, e.g., Draize scoring, prior to the vaccine administration as well as daily following the vaccine administration until the local reaction is resolved. It is recommended that a gross necropsy and complete tissue collection be conducted at study termination after the treatment phase as well as after a recovery phase. A select histopathological evaluation should be done with focus on immune organs, organs that may be primarily affected due to the particular route of administration, pivotal organs and the site of vaccine administration. Full tissue examination would be required in the case of novel vaccines with no prior nonclinical and clinical experience.

4.4. Safety pharmacology

The WHO guideline does not recommend for safety pharmacology studies to be conducted (www.who.int, 2003). This is endorsed by the US. In contrast, the EU guidance requests that safety pharmacology information, e.g., assessment of potential toxic effects on vital organs such as central nervous system, cardiovascular and respiratory systems, be obtained prior to vaccine testing in humans. The rationale for the EU's request is based on experience derived from studies evaluating the effects of pertussis toxin (de Wildt et al., 1982) and *haemophilus* influenza (van Amsterdam et al., 1998). Monitoring of safety pharmacology effects can be incorporated in general toxicity studies, preferably in repeat-dose toxicity studies, as significant effects may be revealed after higher exposure.

In regard to safety pharmacology studies, Japan's approach is closely aligned with those of the EU as Japan requires safety pharmacology studies. Japan has developed a decision tree to inform whether a stand-alone safety pharmacology study would be required or if such evaluation can be incorporated in to a repeat dose toxicity study. (Fig. 1).

4.5. Developmental and Reproductive Toxicity (DART) study

The US has developed a guidance document outlining the criteria and design for developmental toxicity studies for preventive vaccines (www.fda.gov, 2006). Such specific guidance does not exist in the EU or Japan.

4.5.1. Reproductive toxicity study

The guidance states that stand-alone fertility evaluations in males and females as outlined in the document entitled, "*Detection of Toxicity to Reproduction for Medicinal Products*" (ICHS5a) published by the International Conference on Harmonization (ICH) are not routinely required, unless there is specific concern that vaccine formulation might adversely affect male or female fertility (ICH S5A, 1994). In general, histopathological data of the male and female reproductive organs derived from repeat-dose toxicity studies are deemed sufficient. Notably, aspects of female fertility are evaluated as part of the developmental and reproductive toxicity (DART) study, in situations where a DART study is deemed necessary (see below).

4.5.2. Developmental toxicity study/embryo-foetal and perinatal toxicity study

The decision to conduct a reproduction toxicity study in animal models for preventive vaccines is made on a case-by-case basis and depends on the target population and the intended clinical use of the product. Developmental toxicity studies for preventive vaccines are usually conducted when the product is indicated for a population that includes women of childbearing potential. The ICHS5A guidance document provides a useful reference for the design of developmental toxicity studies for drug products (ICH S5A, 1994). The most important feature distinguishing vaccines from drugs or other

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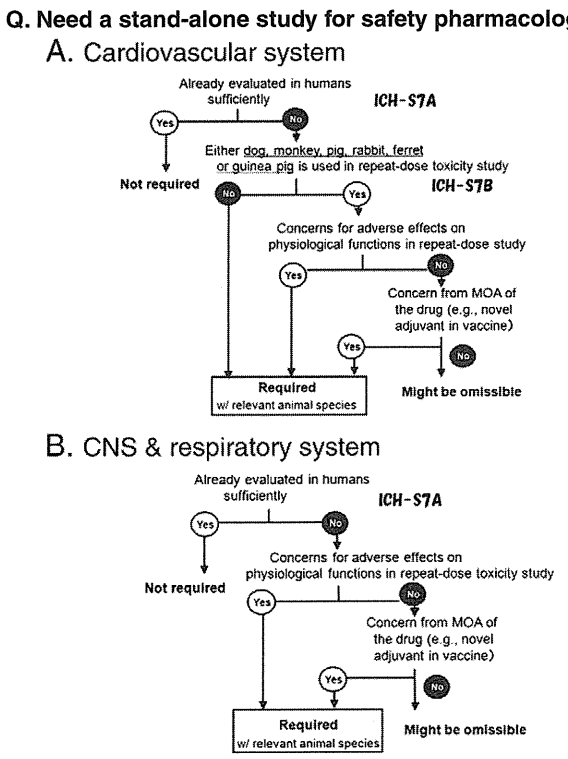

Fig. 1. Japan's decision trees for conducting 'stand-alone' safety pharmacology studies for vaccines. The trees differ depending on which element of the core battery of the cardiovascular system (A), central nervous system (B), and respiratory system (B) is to be evaluated. In either case A or B, ICH-S7A provides the decision criterion whereby a stand-alone study is no longer required for a vaccine that has been already evaluated sufficiently in humans. The history of human administration is based mainly on a supposition of a clinical history outside of Japan. In A, ICH-S7B provides the criterion whereby the animal species specified in the figure need to be used in toxicity studies. Once a decision arises for a stand-alone study, the criteria for choosing relevant animal species for the studies can be found in *Species selection* of this manuscript. Also, stand-alone studies for safety pharmacology need to be completed prior to Phase 1 trials in accordance with ICH-M3(R2). MOA: mechanism of action.

biological products is the desired vaccine-induced immune response. Thus, while the ICHS5A document provides some general guidance for preclinical study designs and endpoints to be evaluated, preclinical testing strategies are frequently modified and specifically tailored to the particular vaccine product under consideration.

For preventive vaccines, the primary concern is any potential untoward effects on the developing embryo and fetus. Therefore, study designs should include subgroups of maternal animals assigned to Caesarian examination at the end of pregnancy for uterine and fetal examinations as well as subgroups allowed to litter and rear their offspring to weaning to monitor the post-natal development of the offspring up to weaning. The use of one relevant animal species, e.g., rat or rabbit, is generally sufficient. To assess potential untoward effects on the pregnant/lactating female and to assess potential adverse effects during the period of organogenesis, the pregnant animal is exposed during the period from implantation to closure of the hard palate and to the end of pregnancy. In general, animals receive a priming dose prior to mating and additional dose(s) during the period of gestation in order to maximize exposure of the developing fetus to the vaccine induced immune response as well as to the vaccine components. Usually, a single dose level that is capable of inducing an immune response in the animal model is assessed. The number of doses administered depends on the onset and the duration of the response. The route of vaccine administration in the animals should attempt to mimic the proposed clinical route of administration. Concurrent control animals should be dosed at the intervals as test group animals. The reproduction toxicity study typically includes a post natal follow up of the offspring from birth to weaning to evaluate normal growth and development and maternal antibody transfer. If the vaccine is formulated with adjuvant, particularly one that is novel in nature, it is recommended to include a group that receives adjuvant alone to evaluate the potential effects of the adjuvant on development in the absence of the vaccine antigen. The endpoints chosen to conduct developmental toxicity studies for preventive vaccines include those traditionally used to evaluate the potential for teratogenic effects as recommended in the ICHS5a document (ICH S5A, 1994). In addition to these endpoints, an assessment of the vaccine induced antibody response should be performed to verify exposure of the embryo/fetus to maternal antibody.

Even though the EU and Japan have not developed specific guidance for DART studies, there is, in general, concurrence among the three regions regarding the principles outlined in the US guidance. However, while the US guidance recommends that DART studies can be conducted in parallel with phase III clinical trials provided that females of childbearing potential enrolled in the clinical trial exercise appropriate measures to prevent pregnancy, the EU and Japan have not developed an official policy regarding the timing of DART studies. These regions follow recommendations made in the ICH-M3 (R2) guideline, which states that embryo-fetal studies (EFD) should be completed prior to phase 3 clinical trials.

4.6. Mutagenicity/genotoxicity and carcinogenicity

These types of studies are generally not needed for preventive vaccines. However, when novel additives (adjuvants, excipients, preservatives) are included in a vaccine, genotoxicity study should be considered on a case-by-case basis and depending on the nature of the adjuvant. This recommendation does not differ among the US, EU and Japan. Notably, a standard battery (ICH2B) of genotoxicity testing is the default position of EU for synthetic products (except for peptides).

4.7. Local tolerance

In many cases, a stand-alone local tolerance study is not necessary. This type of study can be incorporated into single-dose or repeat-dose toxicity studies, in order to reduce animal use. Data on local tolerance should be generated using the clinically intended route of vaccine administration. There is no disconcordance among the EU, the US and Japan.

4.8. Adjuvants

For a novel adjuvant, the potential for local and systemic reactions, including hypersensitivity, etc., should be evaluated in repeated dose toxicity studies. In addition to assessing the safety of the adjuvants by itself, it is also important to assess whether the antigen/adjuvant combination exerts a synergistic effect in the animal model compared to the individual components. There is no disconcordance among the US, the EU and Japan.

4.8.1. The details of CHMP Guideline on adjuvants

In the EU, the regulatory requirement for nonclinical safety studies of the adjuvant alone, as stipulated in the Guidance (EMEA, 2005), is still relevant, especially for a novel adjuvant with no or very limited experience. Toxicity testing should include:

- Local tolerance studies (designed according to the intended route of administration)
- Investigation of the possibility for hypersensitivity and anaphylaxis (antigen-specific IgE induction should be taken as indication for hazard)

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- Pyrogenicity testing (using i.v. route of administration and the rabbit species)
- Systemic toxicity studies (including histological examination of tissues and organs; a full list of histology for novel adjuvants with no prior experience; considering to establish dose-response relationship for result interpretation; dose ranges should reflect clinical dose rather than reach a maximum tolerated dose)
- Reproduction toxicity studies (designed according to clinically intended dosing schedule)
- Genotoxicity (for synthetic adjuvants, require a standard battery of testing (ICH 2B) as EU default position, may not be relevant for biological adjuvants including peptides); carcinogenicity studies are not required.

Toxicity studies need to follow the pattern of clinically intended use of the vaccine (e.g., route, dosing schedule, maximal clinical dose) and should be conducted in two species (one rodent and one non-rodent species) unless otherwise justified. When considering the species, the species of choice should be one that responds to the antigen with which the adjuvant is intended to be used and, ideally, should be the same as for the 'proof-of-concept' studies. Consideration should be given to the choice of species that reflects the pathophysiology of the human diseases. If an adjuvant can be proven to be species-specific, testing of safety in a single species should be sufficient.

The nonclinical safety studies are also required on the adjuvant–antigen combination (i.e. complete vaccine formulation) and these should be considered in line with the *Note for Guidance on Preclinical Pharmacological and Toxicological testing of Vaccines (CPMP/SWP/465/95)*. Specific attention should be given to:

- Local tolerance: exploring optimal dose ratio
- Repeat-dose toxicity study: reflecting the clinically intended schedule, with dosing number higher than planned for human use
- Characterization of immune response: conducting dose–response studies with different doses of adjuvant combined with different doses of vaccine antigen, using the antigen alone or antigen combined with a well-established adjuvant as suitable controls.

If the adjuvant consists of several components, safety data on each individual component should be provided, in addition to the set of toxicity data on the combination. Toxicity studies with separate constituents might be seen as pilot studies. A toxicity study with final combination should be done under GLP. If a combination of adjuvants is proposed for a vaccine, the rationale for this choice should be provided based on the experimental data.

5. Section C: concluding remarks

5.1. EU

The EU regulatory environment consists of its legal classification and definition for vaccine products in Regulation, the highly-interactive regulatory network including EMA as a main body that contributes to timely release of comprehensive guidelines, and several EU default positions in the requirements for toxicity testing, such as two-species request for toxicity studies with adjuvant alone, a standard battery of genotoxicity testing for synthetic adjuvants, and the need for information on safety pharmacology.

The vaccine guidelines allow a flexible approach for safety assessment of this class of products, because of their high diversity regarding product type, formulation and mode of action, bearing in mind the interspecies differences in immune systems and pathophysiology of the disease. Thus, toxicity requirements are based on evidence, and the extent of data expected will rely upon the level of information available. It should be stressed that the use of the most relevant test models and careful design of the testing program will enable the most predictive nonclinical safety assessment.

The actual regulatory approach continues to encourage the integral assessment principle, that is, including immunogenicity endpoints in toxicity studies. There is no need for strict separation of pharmacology from toxicity testing, taking account of reduction of animal use as well as interpretation of toxicity data.

However, it should be recognized that regulatory toxicity requirements are evolving and may be revised in the future, based on science.

5.2. US

Regulations contained in Title 21 of the Unites States Code of Federal Regulations require information about toxicology testing of biological products including preventive vaccines to support that it is reasonably safe to proceed to a proposed clinical product investigation. Thus, nonclinical safety studies for preventive vaccines are a key component in vaccine development as they aid in establishing the safety of the product to allow entry into clinical trials. The guidance document published by the WHO entitled "WHO guidelines on nonclinical evaluation of vaccine" reflects the current approaches to nonclinical safety evaluation of vaccines that allow flexibility in testing requirements. It should be recognized that gaps remain between currently available tools to assess potential toxicity(ies) of preventive vaccine and the fully relevant preclinical safety evaluations. Thus, as further experience is gained by performing nonclinical safety studies and new methods are developed to assess vaccine safety, approaches to toxicity assessment for vaccines will continue to evolve and will need to be optimized to evaluate product safety, to prevent unnecessary use of animals and to support product development.

5.3. Japan

In response to the nationwide desire to close the 'vaccine gap' between Japan and other countries, two government-funded projects were begun in 2004 to establish guidelines for preventive vaccines against infectious diseases. Based on the outcomes of these research projects, the nonclinical as well as clinical guidelines for vaccines were released on May 27, 2010, the former of which was entitled 'Guideline for Nonclinical Studies of Preventive Vaccines for Infectious Diseases'. Although the Japanese guidelines have some commonalities with non-Japanese guidelines, in general, they have stricter criteria for establishing vaccine safety. One unsolved issue lies in the categorization and testing paradigm of adjuvants. In Japan, the regulatory position for adjuvants has not yet established. Adjuvants are currently defined and evaluated as additives. However, the emergence of novel adjuvants that have mechanism of actions beyond those for traditional additives has led to regulatory challenges including the type of regulatory submissions to be used. In addition, guidelines that specifically address assessments of adjuvants are lacking. Discussions to solve this issue should be useful also for other vaccine types that involve adjuvants, such as therapeutic vaccines.

5.4. Discussion of common issues and current challenges

Nonclinical safety studies using animal models are an important tool to assess the nonclinical safety of preventive vaccines. Several different animal species including mice, rats, rabbits and ferrets are commonly used for the pre-clinical safety evaluation of vaccines and, in some situations, non human primates. However, to date, there are no relevant models that can reliably predict the risk of a vaccine, or an ingredient in a vaccine, that may cause specific adverse events in the clinic. The animal model is usually selected by an *in vivo* demonstration of pharmacological activity, i.e., an induction of antibody in the animal. Ideally, the animal model chosen for the nonclinical safety assessment should be susceptible to the pathogen against which the vaccine antigen is directed. However, many of the

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currently used animal toxicology species are not permissive to the human pathogens. Therefore, differences in the expression and recognition of the vaccine antigen and, for live vaccines, differences in the replicative potential as well as the pathophysiology may be observed in the animal model compared to humans.

In recent years, vaccine antigens have been combined with novel adjuvants to enhance the immune response induced by the vaccine antigen. The nonclinical safety assessment of these products is hampered by the fact that some of these novel adjuvants exert species-specific effects. These may be due to differences in innate immune receptor distribution between animal *vs* human, differences in the cytokine repertoire contributing to local and systemic reactions, etc. In some cases, e.g., if the adjuvant consists of a cytokine itself, the use of species-specific homologues of these biologics for testing in animals may be considered. However, differences in species-specific receptor distribution, the fact that the vaccine formulation used in the clinic will differ from the one used in the animal model and lack of historical data will limit the interpretation of the data derived from such studies. Furthermore, the immunological parameters that are evaluated as part of nonclinical safety assessments of vaccines/adjuvant formulations are generally restricted to vaccine antigen induced immune responses, e.g. antibody levels. However, to fully understand the safety of these products, it is not only critical to discern the pharmacodynamics of the product but also to understand how potential exaggerated pharmacodynamic effects may lead to toxicities. Thus, the incorporation of additional immune markers, such as cytokine levels, in particular for evaluating adjuvant specific immune responses as well as incorporation of other biomarkers (e.g., C-reactive protein and fibrinogen) may be helpful. Also, an understanding of the mechanism of action of the adjuvant used may help assessing the risks including the possibility of triggering or exacerbating potential immune disease events.

Nonclinical safety evaluations are usually conducted using healthy adult animal models. As vaccines may be developed for specific subpopulations (e.g., elderly pediatric and immunosuppressed subjects), the question has been raised whether the safety of vaccine in these populations should be studied in specific animal models, e.g., juvenile models. While some of these models are in early development, they are often not available for nonclinical safety assessments. In addition, interpretation of findings derived from such models presents with challenges, especially since it may not be possible to extrapolate immune system developmental stages from the animal model to humans. Furthermore, it has been suggested to assess the risk of auto-immune responses by conducting studies in auto-immune disease animal models, however, the use of animal models of disease in nonclinical safety assessments of prophylactic vaccines is, to date, exploratory. Lack of historical data, the potential for confounders due to the disease itself present some of the concerns regarding use of such models in nonclinical safety assessments.

The primary purpose of developmental toxicity studies in animal models for vaccine products is to serve as signal for the detection of potential developmental hazards in humans. However, factors that may complicate risk prediction include the species specificity of the induced immune response, species-specific differences in developmental time lines, species-specific differences in anatomy and physiology of reproductive organs, and differences in the dosing regimen between various species, etc. In addition, current endpoints used in developmental toxicity studies may not sufficiently address potential adverse effects of the vaccine antigen and the vaccine induced immune response on the physiology, immune system, and development of the offspring. However, lack of validated assays and lack of animal model(s) that resemble human pregnancy present challenges with respect to what can be assessed to date.

In summary, the selection of an animal model to address the nonclinical safety of vaccines as well as the relevance of the selected animal model to predict toxicities in the clinical presents one of the challenges in vaccine development. In order to reduce, replace and refine the use of animals in nonclinical safety assessments animal models will need to be developed that reliably predict vaccine/adjuvant associated risks in humans. In addition, research is needed to develop validated *in vitro* assays and biomarkers for measuring vaccine activity and toxicity at an early stage in product development to improve vaccine-specific safety evaluation. In doing so, scientific challenges need to be addressed such as clarifying how a response obtained from a cell-based assay has relevance to an entire organism and how the strength of evidence from mechanism-based assays can be used to predict toxicity.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.vascn.2012.01.002.

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平成23年度分担研究報告書

医薬品・治療薬の有効性及び安全性に係わる製造・品質管理・評価技術に関する非臨床研究
－原薬の開発と製造－

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研究要旨
原薬の開発と製造に関するICH Q11ガイドラインは、2011年５月postal sign offでステップ２に達し、その後各極でのパブリックコメントを経て、同年11月セビリア会合とその後の電話会合の後、2012年３月ステップ４合意文書を完成した。本報告書ではステップ４作成に際して検討した主要な論点のいくつかを紹介する。本ガイドラインの完成で2003年７月ブラッセルGMPワークショップから始まったQbDに基づく医薬品開発に関する一連のガイドラインは完成した。今後はガイドラインを円滑に実施するための活動が重要である。

キーワード：原薬開発、製造プロセス、管理戦略

A．研究目的

本ガイドラインは、原薬の製造と開発の方法論及びCTD第３部S.2「製造」に提供すべき事項を示すことを目的として作成された。ICH Q8-Q10の原則（科学とリスクにマネジメントに立脚した体系的開発（QbD））を原薬の開発に適用し、QbDによる開発は規制の弾力的な運用の基盤となることを改めて述べている。原薬開発の２通りの方法（Traditional vs QbD）およびその組み合わせた開発方法をいずれも許容している点はQ8と同様であるが、バイオ医薬品と化学合成医薬品を対象（複雑性が異なるが、共通の基盤を有する）としている点でユニークなガイドラインである。

第１回EWG会合が2008年６月に米国ポートランドで開催され、2010年11月第６回福岡EWG会合でドラフト５を作成、さらに電話会議によりステップ２文書を完成し、2011年５月postal sign offに達した。ステップ２合意文書に到達するまで３年を要した。Q8と異なり製造やバリデーションも対象としたこと、化成品とバイオ医薬品を共にスコープとしたことから、議論すべき事項が拡大したことが長期化した要因である。

日本では、６月28日にステップ２文書が翻訳、事務連絡され、８月15日まで本ガイドライン案のパブリックコメントを実施した。その間８月５日にガイドライン案の説明会をEWGメンバーが講師となり開催し、ガイドラインの周知に努めた。

我が国は、翻訳関連事項も含め590個のパブリックコメントを収集した。一方、米国は430、欧州は480のコメントが寄せられた。収集されたコメントの内訳は、製造工程開発に関するコメントがもっとも多く約30%を占め、次いで出発物質及び生物起源材料の選定に関するコメント、さらに図解例、管理戦略、ライフサイクル等の順であった。これらのコメントを各極はあらかじめ評価し、EWGで検討すべき課題を約200に絞り込み、2011年11月スペインセビリアで開催された第７回EWG会合で検討し、プレステップ４文書を作成した。

セビリア会合後、なお継続検討課題とされた項目に関して電話会議を重ね、３月６日電話会議でステ