なった製品を臨床試験で用いようとする場合は、毒性試験が必要かどうかを決定するために、その製品の調製に関する情報が重要である。生薬製剤を販売されている製品と別の方法で調製する場合（茶のような水による調製の代わりにアルコール抽出）、または別の経路で投与される場合は、臨床試験に用いる抗がん薬剤用の標準的な毒性試験が必要である。生薬治療薬剤の開発は大規模な臨床試験で継続されるため、薬剤投与による病理組織学所見、血清生化学、血液学、生殖、および遺伝的作用を含む動物データを、生薬製剤の個々の成分に関する文献データから、または毒性学試験から入手しなければならない。

長期療法に関する考察

化学予防剤

化学予防剤の非臨床開発は既に説明されており、多くの非腫瘍用薬剤と同様に進められるべきである［32］。主な考察については表 2 にまとめられている。

アジュバント療法

アジュバント療法のために開発された薬剤について必要とされる非臨床試験は、これまでのヒトでの薬剤使用経験、患者に対し予期されるリスクおよびベネフィット、および予測作用機序次第である。最初からアジュバント療法剤としてヒトで検討される薬剤はほとんどない。既に臨床経験がかなり積まれてから、原発腫瘍が切除されているかコントロールされている患者に対する維持治療薬剤として考慮されるのが普通である。しかし、臨床上の用法用量に変更があるかどうかによって、さらに非臨床試験が必要となる場合がある。長期間の臨床使用経験が少なく、がんが再発するリスクが比較的低い患者に対し長期にわたる投与が予想される薬剤については、長期毒性に注目した非臨床試験を実施すべきである。通常，細胞毒性型抗がん剤については，既に多くの臨床経験があること、毎日投与されるのではなく間歇的に投与されること、患者に対するリスクは既に充分理解されていることから、アジュバント療法をサポートするための追加する長期試験は必要ない。実施する場合は、長期試験で目的とするアジュバント療法の投与経路およびスケジュールを用いるべきである。少なくとも臨床治療期間と同じ期間で、げっ歯類では最長 6 ヵ月および非げっ歯類（通常はイヌ）では 12 ヵ月までとする。一連の遺伝毒性試験は，病巣がないと考えられる患者を対象とした臨床試験の前に実施すべきである。がん原性試験は販売許可申請の前までに実施することが望まれる。

ホルモン製剤

ホルモン製剤の作用機序はその他の抗がん剤とはかなり異なっている。このような薬剤は直接的な細胞毒性をもっているわけではなく、抗エストロゲン、プロゲスチン、抗プロゲスチン、アンドロゲン、抗アンドロゲン、アロマターゼ阻害因子、または性腺刺激ホルモン放出ホルモンとして作用する。ホルモン製剤の非臨床毒性評価では、細胞毒性型抗がん剤と同様，臨床療法と同じ経路、スケジュール、治療期間、および原薬の製剤処方を用いる。進行期がん患者による小規模第 I 相臨床試験および第 II 相臨床試験の場合には，薬剤投与を毎日おこなう標準的な 28 日毒性試験が有用である。長期の治療期間による臨床試験は、生存期間が長くなると考えられる患者で計画されるため、追加される非臨床試験は、毒性学的試験の期間は少なくとも臨床試験と同じ長さであるという標準的なやり方に準じる。ホルモン製剤は一般的に長期にわ

たって使用されるため、臓器器官への長期作用に注目する完全な毒性学的評価が必要である。動物における最大投与期間はげっ歯類では6ヵ月、非げっ歯類では12ヵ月である。このような薬剤は一般的に性特異的な適応症について開発されるが、両性による非臨床試験によって、治療対象となる患者群と同じ性の動物で観察される薬剤の一次ホルモン作用とは関連性のない毒性を確認することができる。さらに、その薬剤の非生殖器毒性、感受性、または代謝における性差は異種間で必ずしも相関するとは限らない［6］。両性でホルモン製剤の試験を実施することによって、薬剤投与と関連している可能性のある毒性を把握することができる。

変異原性および染色体異常誘発性を評価する標準的な遺伝毒性試験の組み合わせは、病巣がないと考えられる患者による第Ⅰ相臨床試験前に実施することが望まれる［19,22］。ホルモン製剤を病巣がないと考えられる患者で用いようとしている場合、またはアジュバント療法として用いる場合は、がん原性試験を実施することが望まれる。ラットを用いた生殖能力および受胎能（ICHのステージA-B、SegI）を評価する試験、ラットおよびウサギを用いた催奇形性（ICHのステージC-D、SegII）を評価する試験を実施すべきである。対象患者群およびホルモン療法の期間によって、ICHのステージC-F（SegⅢ）試験も必要となる場合がある。多くのエストロゲン作用薬またはその拮抗薬は構造的にまた薬力学的にジエチルスチルベストロール（DES）と類似している。それは子宮内で曝露された胎児に対して出生後に生殖器の悪性疾患および異常を生じさせることが知られている［25］。DES(diethylstilbestrol)と関連した影響の有無、つまり新生児および性的成熟期の動物の生殖器に変化を生じさせるかどうかの検討が重要であると考えられる［5,31,44,45］。このような試験では、膣腺の疾患などの病理組織像を観察するために、げっ歯類の新生児に3～5日間投与して生殖器の分化発達に注目することも重要である［44,45］。

免疫調整剤

がん細胞に対する体の免疫反応を調整する治療薬剤は、標準的な細胞毒性型抗がん剤でみられるような重度の毒性を動物で生じさせる濃度より遙かに低い濃度でその効果を発揮する。免疫調整剤に対する生物学的反応の幾つかは種特異的で、免疫系に対する毒性と関連している。しかし、種特異的でなく，免疫系の調整とは直接関連しない毒性も発現する。したがって、このような種特異的でない毒性を確認するために実施された標準的な毒性評価が重要である。ホルモン製剤の場合と同様、進行期のがん患者を対象とした第I相臨床試験および第II相臨床試験をサポートするには連日投与の標準的な28日毒性試験で充分である。毒性学的試験に加え、作用機序に関する知識も免疫調整剤の安全性を評価し、初回投与量を決定するのに役立つ。がんを治療するために用いられるその他の薬剤とは異なり、これらの薬剤は望ましい活性に関しベル型用量反応曲線を示す場合があるため、毒性評価に適切な免疫学的反応の測定を組み合わせた試験が特に有益である。したがって、初回投与量は有効治療範囲を超えないことが非常に重要である。免疫調整剤では，第I相臨床試験の早期に投与される低用量では、血漿中濃度が通常の薬物動態試験では測定できないことがしばしばある。薬物動態データの代わりに、有効血漿中濃度に達成していることを示すために臨床で用いることのできる活性での代替評価項目に関する動物データを提供することは有用である。評価されている代替活性マーカーには、インターフェロン、TNF-α、neopterin、またはβ-2-ミクログロブリンの誘導が含まれる[41,49]。

修飾療法に関する考察

多剤耐性阻害剤

治療前、治療中、または再発時に、多くの腫瘍は構造的に関連性のない様々な抗がん剤に対し耐性を獲得する。この現象は多剤耐性（Multidrug resistance :MDR）と呼ばれている。MDRの機序には、P-糖蛋白（P-gp）、MDR-関連蛋白（MRP および LRP など）、トポイソメラーゼ、およびグルタチオン-S-トランスフェラーゼの発現変化などが挙げられるが、これに限定されるものではない。現在開発中の MDR 阻害剤の多くは、P-gp-依存性機序を標的としている。P-gp は *mdr*1 遺伝子によってコード化されるが、これは MDR を発現している腫瘍で増幅されているか過剰発現している［26］。排出ポンプとして機能することにより、P-gp は薬剤の蓄積を低下させ、腫瘍細胞内での抗がん剤の細胞障害性を抑えてしまう。P-gp は多くの正常組織（消化管、脳、腎臓、および肝臓など）でも発現している［9］。P-gp 発現の 1 つの役割は、これらの組織から毒性物質を排出することと考えられる。MDR 阻害剤によって P-gp の排出機能を阻害すると、P-gp を発現している腫瘍組織内の細胞毒性型抗がん剤濃度が上昇する。しかし、阻害することによって、正常な P-gp 発現組織内の細胞毒性型抗がん剤濃度も上昇し、細胞毒性型抗がん剤単独によって生じる毒性の程度および種類が変化する可能性がある［1］。さらに、臨床試験および非臨床試験では、P-gp に影響する薬剤は細胞毒性型抗がん剤の薬物動態を有意に変化させることが示されている［2］。

MDR 阻害剤と細胞毒性型抗がん剤とを併用するとリスクが高まるという観点から、臨床試験での安全性を確認するために次の非臨床試験が重要であると考えられる。第一に、早期臨床試験で考えられる使用期間を考慮し、MDR 阻害剤単独による標準的な毒性試験を実施する。第二に、MDR 阻害剤と細胞毒性型抗がん剤とを併用した試験を 1 種類の動物（通常はげっ歯類）で実施し、細胞毒性型抗がん剤の低毒性用量と重大な毒性を示す用量の両者で毒性を評価する（表 **3**）。この情報は、毒性評価も同時に評価する *in vivo* での併用薬効試験からも得ることができる。これまでの経験に基づき、ある細胞毒性型抗がん剤を用いた併用試験は、その構造に関連性のある分類に属する全ての細胞毒性型抗がん剤を併用した場合の安全性を確認する際に充分利用できる。第三に、併用によって生じる毒性を判断するために薬物動態的変化が重要であることが多いため、適切な薬物動態パラメータも入手すべきである。

抗がん剤と MDR 阻害剤を併用する場合の、初回投与量の選択および増量計画には幾つかの方法がある。研究者の何人かは比較的高用量（または薬効濃度）の MDR 阻害剤を用いて、抗がん剤を増量していく方法を選択している。別の研究者は高用量の細胞毒性型薬剤を用いて、MDR 阻害剤を増量していく方法を用いている。この方法のいずれが優れているかは確認されていない。非臨床試験で、MDR 阻害剤のある場合と無い場合で既定用量の細胞毒性型薬剤の毒性の強さ，または安全係数を算出することによって、いずれの用量選択方法を採るべきか明らかになるだろう。この安全係数を出すため受け入れられる評価項目には、骨髄抑制や死亡などの重度の毒性の直接評価、または血漿中濃度測定などが含まれる。例えば、非臨床試験で阻害剤の治療用量が細胞毒性型薬剤の AUC を 5 倍増加させた場合、単独で使用する際の臨床用量を 5 分の 1 に減らした細胞障害型薬剤の初回投与量が適切と考えられる。細胞毒性型薬剤投与量のさらなる調整（増量または減量）は、初回臨床投与経験に基づいて決定される。

放射線および化学療法増感剤

腫瘍を適応症とする増感剤を開発するためには、補足的非臨床試験が重要となる。増感剤単独を用いた2種類の動物による標準的な毒性試験プロファイルに加え、細胞毒性型薬剤または細胞増殖抑制剤の非腫瘍組織に対する毒性を増感剤が増強する作用に関するデータが強く望まれる。MDR 阻害剤と同様、1 種類の動物（通常げっ歯類）による増感剤と細胞毒性型薬剤を併用した試験で、細胞毒性型薬剤または放射線療法の毒性が最小となる用量と重大な毒性が現れる用量での毒性を評価することが重要と考えられる（表 **3**）。増感剤をある薬剤（L-ブチオニン-*S*、*R*-スルホキシミンおよびアルキル化薬など）と併用する簡単な毒性試験であっても、放射線増感剤での、放射線毒性は病理組織学的検査でのみ現れ、さらにその毒性はかなり遅延して発現することから、通常の試験では把握できないことが多い。放射線増感剤によるこの問題に対処する 1 つの方法は、併用による包括的な毒性試験の代わりにマウスの皮膚および後肢による収縮測定法［43］などがある。しかしこのような動物に対する投与は、予定している臨床試験をサポートするようにデザインされるべきであるが、複数回に分けて照射する放射線療法が臨床で想定される場合には、必ずしも実施可能とは限らない場合もある。

初期臨床試験をいかに効率良く実行できるかは、薬剤併用方法に大きく依存しており、増量およびスケジュールに関してはその薬剤特異的なものである。増感剤を治療効果のある療法と併用する場合は、新しい増感剤の初回投与量、投与頻度、および増量計画について慎重に考慮する必要がある。標準療法のサイクルを著しく短縮させたり遅らせたりすることによる毒性増強が併用によって生じることを避けるべきであり、標準療法の効果は維持しなければならない。受け入れうる 1 つの方法は、放射線または抗がん剤の標準量を投与し、ある程度の活性を発揮するが、ほとんど毒性を生じない量の増感剤を投与することである。その他の方法も、明確な科学的根拠によって裏付けられているならば、受け入れられる。

化学保護

化学保護は抗がん剤の毒性作用を緩和する薬剤を利用することである。このクラスで販売されているものには、デクスラゾキサン、アミフォスチン、メスナおよびロイコボリンなどがあり、それぞれドキソルビシン（心臓）、シスプラチン（腎臓）、イホスファミド（膀胱）およびメトトレキサート（大量投与時の救援療法）の毒性を緩和する。げっ歯類一種および非げっ歯類一種による化学保護剤単独の毒性試験は臨床試験で計画されている用法に基づいて実施される。これらの試験は、抗がん剤と併用される場合と同じ投与経路、投与スケジュール、投与期間によって実施されるのが普通である。催奇形性があるかどうか不明な化学療法剤と併用される場合、保護剤単独による生殖毒性試験も考慮すべきである。化学療法剤が催奇形性をもつことがわかっている場合、保護剤がこの毒性を防ぐことができるかどうかを評価することは有用である。化学保護剤の初回臨床用量は，予測される有効性に基づき選択するのが理想であるが、毒性評価の標準的な基準によって算出される用量（すなわち、非げっ歯類に対し重度の毒性を示さないかぎりげっ歯類の STD_{10} の 10 分の 1）を超えてはならない。

主要な問題は化学保護剤単独での毒性であるが、その他の懸念として、化学療法の抗腫瘍作用を減弱させる可能性、および化学療法薬の毒性作用を増強する可能性が挙げられる。例えば、ロイコボリンはメトトレキサートを過量投与した場合の作用を緩和するが、5-フルオロウラシルの毒性を増強する可能性がある［42］。シスプラチンの毒性を抑えるために検討されたdiethyldithiocarbamateは、ラット *in vivo* モデルで腹腔内投与された場合、化学療法終了後の腫瘍再増殖率を増加させた［3］。臨床試験では、100 mg/m^2 シスプラチンの薬理作用に変化は認められなかったが、シスプラチンにdiethyldithiocarbamateを加えると毒性による患者の離脱が有意に増加した［24］。別の臨床試験で、hexamethylmelamineおよびシスプラチンの神経毒性を抑えるためにピリドキシン（ビタミン B_6）を投与したところ、卵巣がん患者の反応持続期間が有意に短縮した［48］。このような臨床所見は、腫瘍保護作用および毒性の変化について化学保護剤を用いた非臨床試験で検討しなければならないことを示唆している。

化学保護剤と抗がん剤を併用した場合の毒性データは、病理組織学的データがあれば、薬効試験から得ることができる。*in vitro* データは有用であるが、それぞれの薬剤の代謝プロファイルの変化要因などが結果に影響する可能性があるため、化学保護剤による腫瘍保護への影響は *in vivo* で検討しなければならない。抗がん剤単独の場合と抗がん剤および化学保護剤を併用した場合の反応持続期間（腫瘍再増殖までの時間）を比較することは特に重要である。化学保護剤と化学療法薬との相互作用に関するこのような情報は、主要臨床試験または大規模な臨床試験をデザインする際に有益となる。

投与経路または製剤処方の変更に関する考察

抗がん剤の投与経路または製剤処方の変更は、薬剤の有効性を改善するという目標のため進められている。既に静脈内投与によって検討されている薬剤の臨床試験を経口投与で実施しようとする場合は、非臨床試験において、（消化管の細菌叢、腸管壁、または肝臓の初回通過効果による）肝毒性の増強、直接的な消化管毒性、または代謝の変化についてさらに検討しなければならない。動物における生物学的利用率データを含む経口投与毒性試験，または消化管毒性および肝臓毒性の評価を含む経口投与薬効薬理試験は，このような問題の解決に有用である。このような試験で用いられる投与スケジュールには、第I相臨床試験で計画されている投与スケジュールが反映されていなければならない。特に代謝物が活性または毒性に関与していると考えられている場合には、血中に存在する薬剤の形態についても慎重に検討する。静注製剤をヒトに投与した場合の薬物動態データも、経口製剤の初回投与量を決定するのに有用であるが、必須なものではない。

臨床で経口投与されている薬剤について静脈内投与を申請する場合、静脈内投与による全身曝露量の増加，ならびにその結果生じる毒性の増強が主に懸念される事項である。動物における静脈内投与毒性試験（初めの第I相臨床試験で計画されているのと同じ投与スケジュールを設定）または静脈内投与後の曝露量を担保できる経口製剤におけるヒトの薬物動態データが、静注製剤の臨床試験開始前に重要となる。同様に、臨床試験で経口抗がん剤の製剤処方を変更する場合、全身曝露の増加に関しても取り組まなければならない。新しい製剤を使用する際に必要な試験は、最初の製剤におけるヒトの生物学的利用率および新しい製剤における生物学的利用率の有意な増加の可能性によって変わってくる。例えば、最初の製剤によるヒトの生物学

的利用率が 100%近い場合は、新しい製剤によって毒性が増強するリスクは低く、新たな試験は必要ないと考えられる。一方、最初の製造においてヒトの生物学的利用率が低い場合、適切な動物種で新しい製剤と比較する生物学的利用率試験の実施を考慮しなければならない。新しい製剤による初めての第 I 相臨床試験の適切な初回投与量はこれらのデータから予測できる。ある状況では、生物学的利用率の変化を考慮し用量が下げられることにより、動物試験の結果がなくても新しい製剤のヒトにおける検討を実施できる場合もある。

まとめ

非臨床試験は薬剤の開発過程で必須項目である。対象疾患が生命を脅かすものであること、および多くの場合、毒性発現用量をヒトに投与することから、新しい抗がん剤の非臨床開発は独特である。腫瘍学領域では、臨床試験において安全で有効な初回投与量および投与スケジュールを決定する際にこれらの試験は特に有用である。これらの試験は、臨床における毒性およびその回復性の予測にも有用であり、増量計画を決定する方法も得られる。充分な非臨床データがあれば、第 I 相臨床試験で治療効果のない用量またはその臨床試験を受ける患者の数を抑えることができる。また、第 II 相臨床試験の至適用量をより素早く決定することができる。臨床試験で計画されているのと同じ投与スケジュール、投与期間、製剤、および投与経路を用いて実施された非臨床試験は非常に有用である。

基礎研究によって、悪性疾患の根幹となる新しい腫瘍発生、進展における細胞機序に関する情報が提供され続けており、その機序を利用しようとする薬剤開発へと結びついている。新しいクラスの薬剤を開発する最善の方法は、古く既存の抗がん剤が開発時に行われてきた方法とは異なっている可能性がある。新しい生物学的評価項目および新しい方法が毒性試験において開発される可能性もあり、予測は困難である。本報告の提案は、臨床で用いる革新的な治療薬の開発を妨げることのないように，限定的または特異的なものとすることを避け、取り組まなければならない事柄を示した。

薬剤の毒性プロファイルを評価するために実施する試験のほとんどは、GLP に準じていると考えられる［16,17］。進行がん患者による第 I 相臨床試験を開始する前に、通常は 2 種類の非臨床毒性試験が実施される。1 つはげっ歯類による試験で、致死的毒性発現用量および致死的でない毒性の発現用量が確認される。もう 1 つは、非げっ歯類において致死的ではないが重篤または不可逆的な毒性の生じない用量を確認する試験である。これらの試験は、可能な限り、有効性について妥当なスケジュールに基づき、第 I 相臨床試験で計画されているスケジュールおよび期間で実施されるべきである。

要求はされていないが、薬力学的試験および薬物動態試験によって、安全性プロファイル（初回投与量、増量、および併用）および薬剤の適切な使用（がん種、スケジュール、および経路）に関する多くの追加情報が得られる。この情報は、第 I 相臨床試験または第 II 相臨床試験の目的が MTD（最大耐量）を求めることではない非細胞毒性型抗がん剤（MDR 阻害剤および免疫調整剤など）を開発する際に特に重要である。

開発中の抗がん剤の種類によって、初回投与量を推定する方法が異なるのは当然である。一般に細胞毒性型薬剤のヒト第I相臨床試験における初回投与量は，非げっ歯類において非可逆的な毒性が認められないかぎり、げっ歯類の STD_{10} の 10 分の 1（mg/m^2）である。この用量において非げっ歯類で不可逆的な毒性が認められる場合、初回投与量には，非げっ歯類で致死的な毒性または重篤で不可逆的な毒性を生じない最高用量の 6 分の 1 より低用量を設定する。非細胞毒性型薬剤では、初回投与量を選択する際に、重大な毒性を生じないという前提で、薬剤の薬効用量を考慮しなければならない。初回投与量を選択する方法にかかわりなく、計画している増量法はトキシコダイナミクスおよび薬力学の用量反応曲線の特徴、発現した毒性の種類、および薬剤の薬物動態に基づいてデザインされなければならない。

ヒトに対する毒性に関する情報が入手されている抗がん剤の開発後期には、追加の毒性試験の必要性について検討する。多くの細胞毒性型薬剤の場合、限られた期間の毒性試験で充分である。化学予防療法、アジュバント療法、長期ホルモン療法、または長期免疫調整療法に用いられる薬剤の場合は、げっ歯類では最大で 6 ヵ月まで、非げっ歯類では 12 ヵ月までの非臨床毒性試験が、安全性の評価および承認申請の取得のために重要である。さらに、場合によっては（たとえば化学予防療法を目的とする薬剤）、生殖毒性およびがん原性試験を実施する。動物およびヒトでの毒性プロファイルによるが、標的臓器となりうる臓器器官の毒性について評価する特殊試験も有用である。

FDA の CDER の腫瘍製剤部門は、特定の抗がん剤について開発早期の段階で話し合い、迅速で効率のよい医薬品開発が促されることを歓迎している。IND を提出する前に、治験依頼者は適切な FDA 担当者と話し合い、試験計画について前 IND 評価を依頼することができる。こうすることによって、不必要な試験のために時間および資源を費やすことが避けられる。また、期待される新薬の臨床試験を早めることができるであろう。

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校正刷りで加えられた注釈：ICH S4 文書草案「動物を用いた慢性毒性試験の投与期間について（げっ歯類および非げっ歯類の毒性試験）」は、EU、日本、および米国によって検討中である。現在の形で実行される場合は、国際的に開発される医薬品のほとんどで非げっ歯類の毒性試験の最長期間は 12 ヵ月から 9 ヵ月に変更される。

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ORIGINAL ARTICLE

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Regulatory considerations for preclinical development of anticancer drugs

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Abstract The entry of new anticancer treatments into phase I clinical trials is ordinarily based on relatively modest preclinical data. This report defines the battery of preclinical tests important for assessing safety under an Investigational New Drug application (IND) and outlines a basis for extrapolating starting doses of investigational anticancer drugs in phase I clinical trials from animal toxicity studies. Types of preclinical studies for the support of marketing of a new anticancer drug are also discussed. This report addresses differences and similarities in the preclinical development of cytotoxic drugs (including photosensitizers and targeted delivery products), drugs used chronically (chemopreventive drugs, hormonal drugs, immunomodulators), and drugs intended to enhance the efficacy (MDR-reversing agents and radiation/chemotherapy sensitizers) or diminish the toxicity of currently used anticancer therapies. Factors to consider in the design of preclinical studies of combination therapies, alternative therapies, and adjuvant therapies in the treatment of cancer, and to support changes in clinical formulations or route of administration, are also discussed.

Key words Antineoplastic agents · Toxicity tests · Toxicology · Guidelines · Phase I clinical trials

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Introduction

Malignant, nonresectable cancers are life-threatening, and aggressive measures are used in treating them. Antineoplastic therapies frequently include toxic chemicals or biological products that are designed to destroy tumor tissue or halt cell replication. Despite the serious toxicities of many anticancer drugs, careful dosing, clinical monitoring and prompt treatment of toxicity makes the side effects less threatening to a patient than their disease. Since it is recognized that doses of anticancer drugs high enough to kill cancer cells usually induce serious side effects in patients, the preclinical testing of oncology drugs differs from testing of nononcology drugs. The Division of Oncology Drug Products within the Center for Drug Evaluation and Research (CDER) at the US Food and Drug Administration (FDA) recognizes the urgency of development of new anticancer drugs and the need to rapidly move promising agents into clinical studies. This report offers a regulatory perspective on the preclinical development of new anticancer drugs that is intended to clarify the differences from the preclinical testing of nononcology drugs and to describe the data that are important to support human testing and eventual marketing.

The types of preclinical studies expected for support of clinical trials and then marketing of a new drug depend on both the intended use of the drug and the population of patients being studied and treated. In situations where potential benefits are greatest (advanced, life-threatening disease), greater risks of treatment toxicity can be accepted and the required preclinical testing can be minimal. In cases where the patient population is free of known disease (e.g. adjuvant therapy or chemoprevention) the acceptable risks are much less and preclinical evaluation should be more extensive [32]. The toxicities of many modulating agents intended to enhance the efficacy or diminish the toxicity of anticancer agents are more similar to those of

nononcology therapies. However, these modulating agents could enhance the toxicity or diminish the activity of cytotoxic drugs by altering their toxicodynamics, pharmacodynamics, and pharmacokinetics. Thus, toxicological evaluation in combination with the modulated cytotoxic drug is an important part of preclinical development.

The following considerations are offered in an effort to balance the risks to be borne by the proposed patient population and the realities of drug testing in humans. The differences in preclinical testing between cytotoxic, chronic (i.e. adjuvant therapy, chemopreventive drugs, hormonal drugs, and immunomodulators), and modulating therapies are emphasized. Issues of chemistry and manufacturing controls, clinical study design, and development of biologic agents for cancer treatment are beyond the scope of this report. If the appropriate preclinical development strategy remains uncertain after contemplating the following considerations, then sponsors are encouraged to initiate pre-IND discussions with Division staff regarding their preclinical study plan.

General considerations for anticancer drug development

Preclinical studies of anticancer agents

The safety of first-time use in humans is assessed through preclinical studies of pharmacodynamics, pharmacokinetics (toxicokinetics), toxicity, and their relationships. The purposes of these safety studies are: (a) to determine a starting dose for clinical trials that is both reasonably safe and allows for possible clinical benefit for the patient, (b) to identify potential end-organ toxicities and determine their reversibility, and (c) to assist in the design of human dosing regimens and escalation schemes for clinical trials. Animal toxicity studies most effectively accomplish these objectives when performed using schedules, durations, formulations, and routes comparable to those proposed in clinical studies. Use of longer duration preclinical studies may lead to underestimates of the appropriate clinical dose, while shorter studies may not identify cumulative dosing toxicities. The toxicity studies should generally conform to the protocols recommended by the National Cancer Institute for toxicology assessment for anticancer agents[1] and are expected to be conducted in accordance with Good Laboratory Practices (GLP) [16, 17]. When studies are not performed according to GLP, deviations should be documented and the potential impact of these deviations on study outcome and credibility should be described [16, 17].

Typically, only two toxicology studies are essential to support initial phase I clinical trials in patients with advanced cancers (Table 1). The first of these is usually a study in rodents that identifies doses that produce life-threatening and non-life-threatening toxicity. The second study should determine whether doses identified as tolerable in rodents produce life-threatening toxicity in a non-rodent species. At least one of these studies should assess clinical signs, body weight, food consumption, clinical pathology, and gross pathology over a range of doses from nontoxic to toxic and should include an examination of histopathology at doses that cause toxicity (or at the highest dose tested). Genotoxicity tests are not generally needed for cancer chemotherapies to support testing in phase I clinical studies unless healthy volunteers will be entered into the study.

While not essential, information on the pharmacodynamics and pharmacokinetics of drugs is extremely valuable for supporting the safety profile and can significantly contribute to the efficiency of drug development. A phase I study may be conducted with no in vitro or in vivo preclinical pharmacodynamic information, but preclinical studies on biological activity and efficacy can substantially aid in clinical study design. Such studies help estimate effective dosages, dosing schedules, and optimal plasma concentrations. This information is likely to be particularly useful when developing noncytotoxic agents. It may be desirable to develop such agents (e.g. immunomodulators) by escalating the human dose to a pharmacodynamically active range rather than to the maximum tolerated dose (MTD). Pharmacokinetic data can be gathered as a part of pharmacology or toxicity studies and do not usually need to be collected separately. Single- and multiple-dose pharmacokinetic studies in the most appropriate species are best performed using dosing schedules, durations, and routes comparable to those that will be used in clinical studies [15]. The pharmacokinetic information obtained assists the evaluation of animal toxicity and efficacy, and may suggest modifications in the intended dose, route or schedule for the clinical trial. The importance of the parameters being measured will vary depending on the clinical trial design and therapeutic classes as discussed in the subsections below. In combination with pharmacodynamic data, this information can be used to help calculate initial doses in humans that have a greater likelihood of activity without adversely affecting safety, and can contribute to optimal dose escalation in early clinical studies.

The proposed therapeutic indication, the outcome of early clinical development, the nature of toxicities seen in animals and in humans, and the projected duration of clinical treatment all determine the preclinical studies necessary to support a New Drug Application (NDA). In general, for oncology drugs, sponsors should conduct toxicity studies using the same schedule and duration of administration as the intended clinical treatment cycle (Tables 1–3). Cytotoxic drugs used to treat advanced disease rarely need studies with more than 28 days of dosing submitted with the NDA (Table 1). In contrast, for drugs intended for continuous

[1] The Developmental Therapeutics Program; Division of Cancer Treatment, Diagnosis, and Centers; National Cancer Institute (Rockville, MD USA) may be contacted for protocol details

Table 1 Preclinical studies for cytotoxic oncology drugs

Stage	Category	Issues to be addressed	Studies considered important[a]	Studies considered useful
IND	All cytotoxics	Starting dose, end-organ toxicities	Rodent[b] and nonrodent[c] toxicology[d]	
		Genetic toxicity	Genetic toxicity panel[e]	
		Effective concentrations, schedule		Pharmacokinetics, pharmacodynamics
	Modifications for Special Categories			
	Photosensitizer	Systemic toxicity	Toxicology studies in subdued light	
		Phototoxicity		In vivo study with illuminated skin
		Plasma $t_{1/2}$		Pharmacokinetics
	Antibody conjugate	Stability	Stability in plasma	
		Toxicity of drug alone	Toxicology in one species	
		Specificity	Human tissue screen	Activity in cell lines ± target antigen
		Pharmacokinetics		Pharmacokinetics
	Liposomal delivery	Drug product toxicity	Include free drug and blank liposomes in toxicity testing	
		Pharmacokinetics versus free drug	Pharmacokinetics	
	Depots	Drug product toxicity	Include free drug and empty depot in toxicity testing	
		Toxicity to contacted tissues	Histopathology of depot site	
NDA	All cytotoxics		Rodent and nonrodent toxicology[a,g], genetic toxicity, stage C-D teratogenicity[f] in rodents and nonrodents	Targeted special toxicity

[a] In general, the schedule and duration of administration in the toxicology study should mimic the clinical trial
[b] Should determine the dose severely toxic to 10% of the animals (STD_{10})
[c] Should determine toxicity of one-tenth the rodent STD_{10} on a mg/m^2 basis
[d] One study should include histopathology
[e] Only for phase I testing in normal volunteers or patients believed to be disease-free
[f] Should be submitted during development
[g] Studies with more than 28 days of dosing are rarely needed

daily administration such as for chemoprevention, adjuvant therapy, or long-term hormonal or immunomodulation therapy, chronic studies should be conducted up to a maximum of 6 months in rodent and 12 months in nonrodent species (Table 2). International Conference on Harmonization (ICH) stage C-D[2] reproductive toxicity studies in a rodent and a non-rodent species are important components of the preclinical evaluation of anticancer drugs and should be submitted early in development [14].

Carcinogenicity studies are not required for cytotoxic drugs used to treat advanced systemic disease, but can be important in the assessment of drugs intended for chronic use for chemoprevention, adjuvant, or hormonal therapy when patients are likely to have a long survival [18]. The current standard is the 2-year rodent bioassay [47], although alternatives may be suitable [20]. Depending upon the nature of toxicities seen with the drug or drug class in animals and in humans, targeted special toxicity studies to support NDA filing may also be needed. For example, in the development of anthracyclines and platinum drugs, which are known to have cardiotoxic and ototoxic potential, respectively, additional preclinical cardiotoxicity and ototoxicity studies have been useful [11, 28, 36, 46]. In addition, neonatal reproductive toxicology and DNA adducting studies have been useful in the development of antiestrogenic agents [5, 30, 31, 37, 44, 45]. A discussion with FDA staff on the preclinical studies needed for marketing approval for a particular drug is recommended at or before the end of phase II clinical studies.

[2] ICH stage A-B, C-D, and C-F reproduction toxicity studies correspond to the previously designated segment I, II, and III studies which are defined by daily administration of drug, respectively, during the period from premating to implantation, implantation to birth (period of organogenesis), and implantation to sexual maturity [14]

Table 2 Preclinical studies for noncytotoxic, chronically administered oncology drugs

Stage	Category	Studies considered important	Studies considered useful
IND	All noncytotoxic chronic therapy	Rodent[a] and nonrodent[b] toxicology[c,d]	Pharmacokinetics, -dynamics
	Modifications for special categories		
	Adjuvant therapy	Genetic toxicity panel[e]	
	Chemopreventive	Toxicology studies should also define NOAEL, Genetic toxicity panel	Efficacy studies Carcinogenicity[e] Stage A-B reproductive toxicity Stage C-D teratogenicity[e]
	Hormonal	28-day toxicology studies usually suffice for limited phase I/II testing in advanced cancer, genetic toxicity panel[e]	
	Immunomodulator	28-day toxicology studies usually suffice for limited phase I/II testing in advanced cancer, genetic toxicity panel[e], define dose versus immunologic response curve to identify shape (bell-shaped?) and surrogate markers	
NDA	All non-cytotoxic chronic therapy	Toxicology studies of equivalent duration to labeled use up to 6 months in rodents and 12 months in nonrodents, genetic toxicity panel, carcinogenicity[f], stage C-D teratogenicity in rodents and non-rodents	
	Additional for hormonal	Stage A-B reproductive toxicity	Stage C-F reproductive toxicity, neonatal reproductive tract toxicity, DNA adducting (drug specific)
	Additional for chemopreventive	Stage A-B and C-F reproductive toxicity carcinogenicity (always)	

[a] Should determine the dose severely toxic to 10% of the animals (STD_{10})
[b] Should determine toxicity of one-tenth the rodent STD_{10} on a mg/m^2 basis
[c] In general, the schedule of administration in the toxicology study should mimic the clinical trial with a duration as long as the intended clinical study up to 6 months in rodents and 12 months in non-rodents
[d] One study should include histopathology
[e] Expected prior to clinical testing in patients with low risk of cancer recurrence, or testing in healthy volunteers
[f] May be unnecessary depending on intended patient population [18]

Starting doses and dose escalation

As described above, one of the primary goals of preclinical studies is to estimate a safe starting dose for the initiation of phase I trials in humans. The starting dose for clinical trials with cytotoxic drugs for oncology indications has traditionally been one-tenth the dose lethal to 10% of rodents on a body surface area basis (milligrams per meter squared) [23, 29, 35]. Studies that actually measure death as an endpoint, however, are not required so long as the dose range studied includes doses that cause severe, life-threatening toxicity. Thus, the starting dose is generally now chosen as one-tenth of the dose that causes severe toxicity (or death) in 10% of the rodents (STD_{10}) on a milligrams per meter squared basis, provided that this starting dose, i.e. one-tenth the STD_{10}, does not cause serious irreversible toxicity in a nonrodent species [29, 35]. If irreversible toxicities are produced at the proposed starting dose in nonrodents (usually dogs) or if the nonrodent is known to be the more appropriate animal model, then the starting dose would generally be one-sixth of the highest dose tested in nonrodents that does not cause severe, irreversible toxicity[3]. In some cases, rodents or dogs may not be appropriate species because they do not model the relevant human biochemical or metabolic processes. For example, folate pools in rodents greatly exceed those in humans [4], so that rodents are generally inappropriate species for testing antifolates. Also, dogs poorly predict the toxicity of some platinum analogues, and an alternate animal model might be preferred [34]. Knowledge of relevant physiological, biochemical, and pharmacokinetic differences between humans and animal models can help determine the most appropriate species to be used for selecting a starting dose. Whenever feasible, these starting doses should be

[3] This calculation is the same as taking one-third of the toxic dose low (TDL) [29, 35]. We believe the current expression of "one-sixth the highest non-severely toxic dose" is simpler and can be applied to the data more universally than taking, in practice, "one-third the dose which causes toxicity but when doubled does not kill the nonrodents". Frequently, the TDL cannot be technically defined in many studies

Table 3 Preclinical studies for modulators of oncology drugs

Stage	Category	Issues to be addressed	Studies considered important[a]	Studies considered useful
IND	All modulators	Starting dose, end-organ toxicities	Rodent[b] and non-rodent[c] toxicology[d]	
		Genetic toxicity	genetic toxicity panel[e]	
		Effective concentrations, schedule		Pharmacokinetics
	Additional studies for special categories			
	MDR modulator	Combination toxicity	One species at minimally and significantly toxic doses of cytotoxic	In vivo efficacy of combination
		Pharmacokinetic perturbations	Pharmacokinetics	
	Chemosensitizer	Combination toxicity	One species at minimally and significantly toxic doses of cytotoxic	
	Radiation sensitizer	Delayed toxicity to normal tissues		Skin/leg contracture
	Chemoprotection	Combination toxicity, tumor protection	In vivo efficacy of combination with histopathology	
NDA	All modulators		Toxicology studies of equivalent duration to labeled use up to 6 months in rodents and 12 months in non-rodents, genetic toxicity, stage C-D teratogenicity in rodents and non-rodents	Targeted special studies

[a] In general, the schedule and duration of administration in the toxicology study should mimic the clinical trial
[b] Should determine the dose severely toxic to 10% of the animals (STD_{10})
[c] Should determine toxicity of one-tenth the rodent STD_{10} on a mg/m2 basis
[d] One study should include histopathology
[e] Only for phase I testing in normal volunteers or patients believed to be disease-free

calculated from studies using the proposed clinical route, schedule, and duration.

The dose escalation scheme for phase I clinical studies often follows the standard or modified Fibonacci procedure [10]. Examples of other common and acceptable approaches include modified continual reassessment methods [13, 39] and pharmacokinetically guided dose escalation strategies [8]. These alternatives often necessitate a more extensive preclinical evaluation. For example, pharmacokinetic guidance of dose escalation is most effectively applied when: (a) linear pharmacokinetics are observed at drug concentrations spanning the pharmacological and toxicological effects, (b) the area under the drug concentration versus time curve (AUC) at the mouse STD_{10} can be defined, (c) protein binding in mouse and human plasma has been quantified, and (d) it is known whether metabolites contribute to the toxic effects [7, 8, 27, 40]. Although preclinical studies are used to determine the starting dose for phase I clinical trials, the highest doses for oncology drugs are rarely restricted by the doses used in preclinical toxicology studies as long as the toxicities of the new anticancer drug can be readily monitored, are reversible, and sufficiently precede lethality in animals. Instead, the maximum dose is restricted by the toxicity observed in the clinical trial, judged most often using NCI/DCTDC Common Toxicity Criteria [38].

Considerations for specific cytotoxic therapies

Combinations of cytotoxic agents

The evaluation of cytotoxic agent combinations has traditionally been conducted in the clinical setting using an empirical approach. This has generally been successful, but may not be optimal. Preclinical studies provide an opportunity to explore a variety of doses, dose ratios, and schedules to optimize benefit and minimize toxicity. Nonetheless, unless there is reason to believe that synergistic interactions occur that would substantially increase the toxicity of the combination, preclinical testing is not considered essential provided that each agent has been fully evaluated in humans. When synergistic effects may be anticipated such as when one agent interferes with the metabolism or elimination of the other agent or both cytotoxic agents target the same metabolic pathway or cellular function, preclinical testing of the combination is desirable.

Photosensitizers

One class of cancer chemotherapeutic drugs is therapeutically inactive until irradiated with light. These

photosensitizers or phototherapy agents usually form radicals after absorbing light energy that are ultimately responsible for tumor destruction. In photosensitizer therapy, tumor tissues are typically irradiated with laser light. When there is a choice, longer wavelengths of the irradiating light are preferred because they cause less direct tissue damage and because they penetrate more deeply into tumor tissue than shorter wavelengths.

Selective damage to tumor tissue is obtained by directing the activating light to the tumor. In addition, most phototherapy compounds concentrate in tumor tissues more than in surrounding normal tissue when given systemically. This increased concentration of photosensitizer combined with localized irradiation can kill tumor cells with great selectivity. Nevertheless, when these compounds are given systemically they commonly distribute in appreciable concentrations in all tissues and this provides the potential for toxicity. When these drugs accumulate in the eye or skin, patients may suffer irreversible retinal damage or severe phototoxicity similar to sunburn when exposed to ambient light [12]. Thus, it is important to know the plasma elimination half-life (and, if possible, tissue elimination half-lives) in preclinical studies so that the length of time a patient should protect themselves from light can be estimated.

Standard toxicity studies with multiple dose levels should be conducted in subdued illumination to clearly define the systemic toxicities of the photosensitizer. Subdued lighting allows systemic toxicities to be more clearly distinguished from phototoxicities. In addition to these standard toxicity studies, it is beneficial to assess phototoxicity before phase I clinical investigation begins because these drugs can cause prolonged photosensitivity. Acceptable models for these photosensitivity tests are either hairless or appropriately shaved species. The photosensitivity assessment should include toxicity testing as a function of both light dose (total energy) and drug dose and should ideally determine the duration of sensitivity in relation to plasma levels of the photosensitizer. Since a primary concern for the patient is the toxicity related to sunlight exposure, the light source for these tests should have a spectral distribution that approximates sunlight. Frequently, doses that are well below the no observable adverse effect limit (NOAEL) when the animal is housed in subdued light are lethal when the animal is briefly irradiated. Even though the photodynamic effect is expected to affect only tissues that are exposed to the light source, there is concern that photodegradation products could cause distant toxicities. Therefore, these phototoxicity tests usually include standard assessments of clinical signs, clinical pathology, gross pathology, histopathology of major organs, and the reversibility of toxicities. Clinical photodynamic therapy does not routinely involve repeated doses, and thus preclinical studies using daily irradiation during repeat dose testing may not be relevant to clinical safety concerns.

Without light these photosensitizers may not cause genotoxicity in standard tests, but subsequent irradiation may cause considerable damage to the DNA of cells exposed to the compound. Thus, genotoxicity tests are best done with and without light. The assessment of clastogenicity and mutagenicity should be done with increasing compound concentrations at a high light dose, and with increasing light dose (total energy) using broad-spectrum light at high compound concentration. The highest doses of drug of each series of tests should be consistent with international standards [19, 22].

In many cases an effective dose of drugs in this class is nontoxic in subdued light and the starting dose can be chosen based on efficacy studies rather than toxicity studies. This pertains only if the projected efficacious starting dose is lower than the safe dose estimated from the toxicity studies.

Specialized drug delivery

Administration of anticancer drugs as depots, attached to carriers, or in specialized encapsulated forms has the potential for significantly improving efficacy. Advantages of specialized drug delivery may include: (a) specific targeting of the drug to the tumor, (b) minimization of toxic side effects, (c) prolongation of therapeutic drug concentrations, (d) improved delivery of hydrophilic drugs to tumor cytoplasm, and (e) practical administration of very lipophilic drugs. Examples of delivery systems include copolymer implants, human albumin microspheres, monoclonal antibody–drug conjugates, and liposomal encapsulation. Development of anticancer drugs administered via carriers or in depots may necessitate additional preclinical evaluation beyond that of conventional cytotoxic drugs.

For antibody–drug conjugates, the two main safety concerns are the potential for toxicity from abrupt release of the drug and the potential for the antibody–drug conjugate to cause unexpected, specific toxicity in normal human tissues. Studies of the stability of the conjugate in human plasma as a function of the proposed release mechanism (e.g. pH if hydrolytic, glutathione concentration if reductive) help determine the necessity of conducting additional toxicology studies [21]. When additional studies are indicated, using the form of the drug released from the conjugate (i.e. including linker groups) may identify clinically important toxicities. Testing the reactivity of the conjugate with a complete panel of human tissues from at least three different sources is suggested [21]. When the target antigen is not expressed in the tissues of the standard preclinical animal models, a tolerance study in Pongidae apes at a dose that is at least double the planned human starting dose should also be considered. Both the reactivity screen and the tolerance study may reveal sites of potential tissue-specific toxicity, while the standard toxicology studies may define nonspecific toxicities. Specificity studies of binding or cytotoxicity in cell lines with and without an expressed target antigen also help to assess whether there is a significant differential between the toxicity to a targeted and nontargeted tissue. If feasible,

pharmacokinetic studies that distinguish between conjugate, free antibody, and free drug are also highly desirable for interpreting toxicology findings and supporting interspecies comparisons. Selection of a starting dose for clinical study should consider not only the results of the toxicity studies with the conjugate, but also the stability of the conjugate and the potential toxicity of released drug.

With liposomal drugs, standard preclinical toxicology studies of the delivery system, free drug, and the final formulation are important for evaluating a drug product's potential for toxicity. Liposomal formulations usually dramatically prolong systemic exposure. Thus, when repeated doses are to be used clinically, it is especially important to study a similar schedule preclinically because of the potential for drug accumulation. When the delivery system is designed to affect drug absorption, distribution, biotransformation, excretion or target organ accumulation, small changes in the design of the delivery system may have substantial effects on overall toxicity. Conducting the toxicity studies with the final formulation can avoid concerns about such effects. Comparative pharmacokinetic studies of the final formulation versus free drug can be very helpful in suggesting schedules and interpreting changes in the spectrum and severity of toxicities. Occasionally, studies of the empty liposomes plus free drug in combination may also be useful for understanding alterations in efficacy seen with the liposomal preparation. For example, blank liposomes may alter the pharmacokinetics of the free drug in a fashion sufficient for therapeutic gain [33].

Preclinical development of depot formulations generally follows that of liposomal formulations. Additionally, a study of the toxicity of the depot in the tissue or compartment intended to be used clinically should be conducted which includes a histopathologic examination of the adjacent tissues. Initial clinical doses similar to the total dose of the drug previously investigated in humans may be used in the absence of significant changes in toxicity profile for the depot formulation.

Alternative therapies

"Alternative" therapies include both single agents and multicomponent entities derived from plants or animals. Herbal products and tissue or fluid extracts from animal sources intended for the treatment or prevention of cancer or precancerous conditions belong in this category. The identity of the active ingredient of these entities is frequently uncertain. Consistency in taxonomic identification, collection, storage, and processing may pose additional difficulties. A useful initial step is to prepare a batch of the drug product large enough to be sufficient for both initial preclinical and clinical studies. The usual battery of toxicology studies for anticancer agents should be conducted unless there is adequate human safety experience. Since it is difficult to correlate specific drug product components with pharmacologic action, attempts should be made early in the development scheme to control the manufacturing processes to produce consistent batches for subsequent preclinical and clinical study. Further efforts should be made in the later stages of development to identify biologic assays which can be used to assure activity and as release specifications for the marketed product.

Herbal products represent a specialized subset of alternative therapies, as there is often significant human experience with their use. If there is a documented history of use of the herbals or if these preparations are freely marketed in the United States, then no preclinical pharmacology or toxicology is required for initial trials using the marketed product. Submission of data on the traditional use, preparation of the product, and safety profile of any known components of the herbal preparation for the IND is encouraged. When a product different from the marketed version is intended for the clinical trial, information on the preparation of the product to be tested is important in determining whether toxicology studies are necessary. If a herbal product is prepared in a manner different from the marketed product (e.g. alcoholic extraction instead of an aqueous preparation such as tea) or administered by an alternative route, then the standard toxicology studies for an investigational anticancer drug may be necessary. As the development of the herbal therapeutic agent continues in expanded trials, animal data including the histopathology, serum chemistry, hematology, reproductive, and genetic effects of the compound should be obtained either through literature data on the individual components of the herbal product or through toxicologic testing.

Considerations for chronic therapies

Chemopreventives

The preclinical development of chemopreventives has been previously described and should proceed similarly to most nononcology drugs [32]. The key considerations are summarized in Table 2.

Adjuvant therapy

The preclinical studies expected for drugs developed for adjuvant therapy depend on the prior human experience with the drug, the anticipated risks and benefits for the intended patients, and the expected mechanism of action. Few drugs are initially tested in humans in the adjuvant setting. Substantial clinical experience with these drugs is thus usual by the time they are considered for therapy in patients who have had their primary tumor removed or controlled. Nonetheless, further preclinical testing may be needed, depending on whether there are changes in the pattern of clinical use.

Additional preclinical studies that focus on long-term toxicity should be conducted for agents with which there is limited long-term clinical experience and intended for chronic treatment of patients in whom the risk of recurrence of cancer is relatively low. Cytotoxic drugs normally do not need additional long-term studies to support adjuvant use because the clinical experience with these drugs is usually extensive, they are usually administered using intermittent cycles rather than daily dosing, and the risks to patients are already well understood. When conducted, long-term studies should use the intended adjuvant route and schedule for at least as long as the intended clinical treatment duration, up to a maximal duration of 6 months in rodents and 12 months in nonrodents (usually dogs). A complete battery of genetic toxicity tests should be conducted prior to trials in patients believed to be free of disease. Carcinogenicity studies are usually expected prior to application for market approval.

Hormonal drugs

The mechanism of action of hormonal drugs differs significantly from that of other antineoplastic agents. These drugs are usually not directly cytotoxic, but may act as antiestrogens, progestins, antiprogestins, androgens, antiandrogens, aromatase inhibitors, or gonadotropin releasing hormone agonists. As with cytotoxic therapies, the preclinical toxicity assessment of hormonal drugs should use a similar route, schedule, duration of treatment, and formulation of drug substance as that proposed in clinical therapy. Standard 28-day toxicology studies with daily drug administration usually support small, phase I and phase II clinical trials with advanced-stage cancer patients. As clinical studies with longer durations of treatment are planned in patients likely to have an extended survival, additional preclinical testing usually follows the standard practice that the duration of the toxicology study be at least as long as the clinical trial. Because hormonal agents are generally used over an extended period, the complete toxicology assessment may need to focus on long-term effects on organ systems. Maximal duration of treatment in animals is usually limited to 6 months in rodents or 12 months in nonrodents. Although these agents are customarily developed for sex-specific indications, preclinical testing of both sexes allows identification of toxicities unrelated to the primary hormonal action of the drug that may be obscured in animals of the same sex as the intended treatment population. In addition, sex-based differences in the nonreproductive organ toxicities, sensitivity, or metabolism of a given drug may not be correlated across species [6]. Testing of hormonal agents in both sexes is thus more likely to provide the full spectrum of potential toxicities associated with a drug's use.

It is expected that the standard battery of genotoxicity tests assessing mutagenicity and clastogenicity will be conducted prior to phase I testing in patients believed to be disease-free [19, 22]. Carcinogenicity studies are expected if the hormonal drug is intended for use in patients believed to be disease-free or as adjuvant therapy. Studies should be conducted to evaluate reproductive performance and fertility in rats (ICH stage A–B, segment I), and teratogenicity in rats and rabbits (ICH stage C–D, segment II). Depending on the patient population and the duration of hormonal therapy, ICH stage C–F (segment III) studies may be needed. Many estrogen agonists or antagonists are structurally or pharmacodynamically related to diethylstilbestrol (DES), which is known to cause reproductive tract malignancy and abnormalities in humans exposed in utero [25]. Testing the potential of compounds related to DES to cause reproductive tract changes in neonates and pubescent animals is therefore considered important [5, 31, 44, 45]. Such studies typically focus on reproductive tract development following 3–5 days of dosing in rodent neonates in order to observe such pathologies as vaginal adenosis [44, 45].

Immunomodulators

Therapeutic agents that modulate the body's immune response to cancerous cells usually do so at concentrations significantly lower than those that cause severe toxicities in animals of the type seen with standard cytotoxic agents. Some biological responses to immunomodulators are species specific and may be related to toxicity to the immune system. Non-species-specific toxicities, however, do occur that are not directly related to modulation of the immune system. Thus, a standard safety evaluation conducted to identify these non-species-specific toxicities is important. As for hormonal agents, standard 28-day toxicology studies with daily drug administration are adequate to support initiation of phase I and phase II clinical trials that enroll advanced-stage cancer patients. In addition to the toxicology studies, knowledge of the mechanism of action also contributes to the evaluation of the safety of immunomodulators and selection of a starting dose. Studies that combine a measurement of the appropriate immunological response in addition to toxicity assessments are particularly useful because these agents, unlike most other drugs used to treat cancer, have sometimes exhibited bell-shaped dose response curves for desired activities. It is therefore especially important to use a starting dose that does not exceed the beneficial therapeutic range. The low doses often administered early in the phase I study sometimes give plasma concentrations of immunomodulators that preclude conventional pharmacokinetic study. In lieu of pharmacokinetic data, it may be useful to provide animal data on possible surrogate endpoints of activity that can be used in the clinic to demonstrate that active concentrations have been reached. Surrogate markers of activity that have been assessed include induction of interferon, TNF-α, neopterin, or β-2-microglobulin [41, 49].

Considerations for modulating therapies

Multidrug resistance-reversing agents

Prior to therapy, during therapy, or at the time of relapse, many tumors develop resistance to a variety of structurally unrelated anticancer drugs. This phenomenon is termed multidrug resistance (MDR). Mechanisms of MDR include, but are not limited to, altered expression of P-glycoprotein (P-gp), MDR-associated proteins (e.g. MRP and LRP), topoisomerases, and glutathione-*S*-transferases. Currently most MDR-reversing agents under development target the P-gp-dependent mechanism. P-gp is encoded by the *mdr*1 gene that is often amplified or overexpressed in MDR-manifesting tumors [26]. By functioning as an efflux pump, P-gp causes decreased drug accumulation and reduced cytotoxicity of anticancer drugs in tumor cells. P-gp is also expressed in many normal tissues (e.g. in the gastrointestinal tract, brain, kidney, and liver) [9]. One role of P-gp expression is presumably to carry out the efflux of toxic substances from these tissues. The inhibition of the efflux function of P-gp by a MDR-reversing agent increases intracellular concentrations of the cytotoxic drug in tumor tissue expressing P-gp. However, inhibition may also increase levels of cytotoxic drugs in normal P-gp-expressing tissues, potentially resulting in alterations of the severity and types of toxicities usually associated with the cytotoxic drug alone [1]. Furthermore, clinical and preclinical studies have shown that drugs interacting with P-gp can significantly alter the pharmacokinetics of cytotoxic drugs [2].

In view of the added risks associated with the combination of a MDR-reversing agent and a cytotoxic drug(s), the following preclinical studies are considered important for determining the safety of a proposed clinical trial. First, a standard profile of toxicology studies for the MDR-reversing agent alone should be conducted which take into consideration the likely duration of use in early clinical trials. Second, a study of the MDR-reversing agent combined with the cytotoxic drug in one species (usually a rodent) should be conducted to assess toxicity at both minimally and significantly toxic doses of the cytotoxic agent (Table 3). This information may also be derived from in vivo combination efficacy studies when an assessment of toxicity has been included. Based on experience to date, a combination study with one cytotoxic drug from a structurally related therapeutic class generally suffices for determining the safety of the modulator with all cytotoxic drugs in that class. Third, appropriate pharmacokinetic parameters should be derived since pharmacokinetic changes have often been shown to be important in interpreting the toxicity from such combinations.

There are several approaches for the selection of starting doses and escalation schemes for combinations of anticancer drugs and MDR-reversing agents. Some investigators have chosen to use a relatively high dose (or effective concentration) of MDR reverser and to escalate the anticancer drug. Others have started with a relatively high dose of the cytotoxic drug and escalated the MDR reverser. Neither of these approaches has been established as superior. Preclinical studies can guide either approach to dose selection by establishing a ratio of toxicity or potential toxicity of a given dose of the cytotoxic drug in the presence and absence of the MDR reverser. Acceptable endpoints for establishing this ratio might include direct measures of severe toxicity, such as marrow suppression or lethality, or measures of plasma concentrations. For example, when a therapeutic dose of a reversing agent increases the AUC of the cytotoxic drug fivefold in a preclinical model, a starting dose of the cytotoxic drug that is decreased by a factor of five from the accepted clinical dose of the cytotoxic drug alone would usually be appropriate. Further adjustments in the dose of the cytotoxic drug, either up or down, can then be derived from the initial clinical experience.

Radiation and chemotherapy sensitizers

Additional preclinical studies are usually important for the development of sensitizing agents for oncologic indications. In addition to the standard profile of toxicology studies in two species for the sensitizer alone, data on the ability of a sensitizing agent to enhance the toxicity of a cytotoxic or cytostatic therapy to non-neoplastic tissue is highly desirable. As for MDR-reversing agents, a study of the sensitizer combined with the cytotoxic therapy in one species (usually a rodent) that assesses toxicity at both minimally and significantly toxic doses of the cytotoxic agent or radiation therapy is considered important (Table 3). Although this is a straightforward toxicology study when the sensitizer is combined with a drug (e.g. L-buthionine-*S,R*-sulfoximine and alkylating agents), it is not so simple with radiosensitizers because radiation toxicity may only be apparent upon histopathologic examination and because the toxicity can be substantially delayed. One approach to address this issue with radiosensitizers is to conduct skin and leg contracture assays in mice [43] in lieu of comprehensive toxicology studies of the combination. The dosing scheme for these animals should be designed to support the planned clinical trial, but given the common clinical use of highly fractionated radiotherapy, this may not always be feasible.

How best to conduct the initial clinical trial is highly dependent on the combination modality, and advice on dose escalation and scheduling is product-specific. When the sensitizer is intended for combination with a therapy that has curative potential, the starting dose, frequency of dosing, and dose escalation plan for the new sensitizer needs to be carefully considered. Enhanced toxicities from the combination that significantly shorten or delay cycles of the standard therapy should be avoided so that efficacy of the standard therapy is maintained. One

accepted approach is to administer a full standard dose of radiation or anticancer agent, and a dose of the sensitizer projected to have some activity but that imparts little toxicity to the treatment regimen. Other approaches may also be acceptable provided that they are supported by a sound scientific rationale.

Chemoprotection

Chemoprotection is the use of drugs to mitigate the toxic effects of antineoplastic compounds. Marketed examples of this class include dexrazoxane, amifostine, mesna and leucovorin, which decrease the toxicities of doxorubicin (heart), cisplatin (kidney), ifosfamide (bladder) and methotrexate (high dose rescue), respectively. The toxicologic testing of the chemoprotective agent alone in one rodent and one nonrodent species should be based on the proposed use in the clinical trials. Usually these studies are done with a similar route, schedule and duration of administration as when combined with the antineoplastic agent. Reproductive toxicity testing for the protectant alone should be considered when the protectant is to be combined with a chemotherapeutic agent not known to be teratogenic. When the chemotherapeutic agent is known to be teratogenic, it may be useful to assess the ability of the protective agent to prevent this toxicity. The initial clinical dose for a chemoprotectant should ideally be chosen based on projected efficacy, but should not exceed the dose selected by standard toxicity criteria (i.e. one-tenth the rodent STD_{10} unless that dose is severely toxic to nonrodents).

While the primary issue is the toxicity of the chemoprotective agent alone, additional concerns include the possibility of protection of the tumor from the antineoplastic effects of chemotherapy and the possible augmentation of some of the toxic effects of the chemotherapeutic agent. For example, leucovorin, while able to mitigate the effects of an overdose of methotrexate, can also increase the toxicity of 5-fluorouracil [42]. Diethyldithiocarbamate, investigated to decrease the toxicity of cisplatin, actually increased the rate of tumor regrowth following the end of chemotherapy in a rat in vivo model when administered intraperitoneally [3]. In a clinical study, no change in response to 100 mg/m^2 cisplatin was noted, while the patient withdrawal due to toxicity was increased significantly in the diethyldithiocarbamate plus cisplatin arm [24]. In another clinical study, treatment with pyridoxine (vitamin B_6) to reduce the neurotoxicity of hexamethylmelamine and cisplatin was associated with a significant decrease in duration of response in ovarian cancer patients [48]. These clinical findings emphasize that the potential for tumor protective effects and changes in toxicity should be examined in preclinical studies of chemoprotective agents.

Toxicity data on the combination of the chemoprotectant and the antineoplastic agent can be derived from efficacy experiments, provided that histopathologic data are collected. Although in vitro data are useful, the influence on tumor protection by the chemoprotectant should be examined in vivo, where additional factors such as changes in metabolic profile of either drug may affect outcome. Comparisons of the duration of response (i.e. time to tumor regrowth) between an antineoplastic alone and the combination of antineoplastic and chemoprotectant are particularly important. This information on the interaction between the chemoprotective agent and the chemotherapeutic agent can be valuable for the design of pivotal or large scale clinical studies.

Considerations for changes in route or formulation

Changes in the route of administration or in the formulation of anticancer drugs are often pursued with a goal of improving drug utility. If a clinical trial is proposed by the oral route for a drug that has already been investigated by intravenous administration, then additional preclinical studies should address whether there is enhanced liver toxicity, direct gastrointestinal toxicity, or altered metabolism (due to microflora in the gastrointestinal tract, the intestinal wall, or a first-pass effect through the liver). An oral animal toxicity study with bioavailability data or an oral animal efficacy study with assessment of gastrointestinal and liver toxicity can address these concerns. The schedule of administration used in such a study should reflect the planned schedule of administration in the proposed phase I clinical study. A careful assessment of the forms of the drug present in blood should also be attempted, particularly if it is believed that metabolites contribute to the activity or toxicity. Pharmacokinetic information in humans for the i.v. formulation would also be useful for determining the starting dose of the oral formulation, but is not mandatory.

When i.v. administration is proposed for a drug with which there is oral clinical experience, the main concern is that the systemic exposure and resulting toxicity may be much greater by the i.v. route. Either an i.v. animal toxicity study (using the same schedule of administration as proposed for the initial phase I trial) or pharmacokinetic data with the oral formulation in humans that supports an acceptable exposure after the i.v. administration is important before beginning a trial with an i.v. formulation. Similarly, concerns about increased systemic exposure should be addressed when the formulation of an oral anticancer agent in clinical trials is changed. The studies needed to support use of the new formulation depend on the bioavailability of the original formulation in humans and on whether the potential exists to significantly increase bioavailability with the new formulation. For example, if the bioavailability in humans of the original formulation is near 100%, then there is little risk of increased toxicity with the new formulation and no new studies would be needed. On the other hand, if the bioavailability of the original formulation in humans is low, then a bioavailability

study comparing the new formulations in an appropriate species should be considered. An appropriate starting dose for the initial phase I trial with the new formulation can be projected from these data. In some circumstances, it may be sufficient to test a dose of a new formulation in humans without an animal study, so long as the dose is reduced to take into account potential changes in bioavailability.

Summary

Preclinical studies are an essential component of the drug development process. The preclinical development of new anticancer drugs is unique because of the life-threatening nature of the disease and because in most cases humans will be dosed to toxicity. In oncology, these studies are particularly useful in determining potentially safe and effective starting doses and schedules for a clinical trial. These studies also help to predict clinical toxicities and their reversibility, and provide a means for the determination of a dose-escalation scheme. The availability of adequate preclinical data can minimize the number of patients treated with ineffective doses or therapies in phase I trials and allow rapid determination of phase II doses. Preclinical studies are most useful when conducted using the same schedule, duration, formulation, and route of administration as that proposed in the clinical trial.

Basic research continues to provide information about new cellular mechanisms central to malignancy and often leads to drugs that attempt to exploit those mechanisms. The optimal development of a new class of drugs may differ from successful approaches used in the development of older well-established classes. New biological endpoints and new methods in toxicology may also be discovered and cannot be anticipated. The recommendations in this report have thus attempted to avoid being so restrictive and specific as to impede the development of innovative therapeutics for clinical use. Instead, the concerns that should be addressed have been emphasized.

It is assumed that most of the studies conducted to assess the toxicity profile of a drug follow GLP [16, 17]. Before a phase I clinical trial is initiated in patients with advanced cancers, two preclinical toxicity studies are usually conducted. One is a study in a rodent species that can identify doses that result in life-threatening and non-life-threatening toxicities. The other is a study to confirm that doses are identified that are not lethal and do not cause serious or irreversible toxicity in a nonrodent species. These studies, to the extent feasible, should be based on a rational schedule for efficacy and mimic the schedule and duration proposed in the phase I clinical trial.

Although not required, pharmacodynamic and pharmacokinetic studies can provide substantial additional support for the safety profile (starting dose, escalation, and drug combinations) and optimal potential use of the drug (tumor type, schedule, and route). This information is especially important in the development of noncytotoxic drugs (e.g. MDR reversers and immunomodulators) where the objective of the phase I or II clinical study may not be to reach MTD.

Depending on the type of antineoplastic agent under study, different approaches for estimating starting doses are appropriate. The phase I starting dose for cytotoxic agents in humans is generally one-tenth of the rodent STD_{10} on a milligrams per meter squared basis so long as this starting dose does not cause serious irreversible toxicities in nonrodents. If this dose causes irreversible toxicities in nonrodents, then the starting dose should be no more than one-sixth of the highest dose that does not produce lethality or serious irreversible toxicity in the nonrodent species. For noncytotoxic agents, starting dose selection should take into account the drug's pharmacodynamically active doses, provided that they do not cause substantial toxicity. Regardless of the method used to select the starting dose, the planned dose escalation scheme should be designed based on the slopes of the dose response curves for toxicodynamics and pharmacodynamics, the types of toxicities observed, and the pharmacokinetics of the drug.

In the later stages of anticancer drug development, when information is available on toxicity to humans, the need for additional toxicology studies should be evaluated. For most cytotoxic drugs, toxicity studies of limited duration suffice. With drugs intended for chemoprevention, adjuvant therapy, long-term hormone therapy, or long-term immunomodulator therapy, animal toxicity studies up to a maximum of 6 months in rodents and 12 months in a nonrodent species may be important for assessing safety and for supporting marketing approval. In addition, reproductive toxicity and carcinogenicity studies should be conducted when appropriate (e.g. for chemoprevention indications). Depending upon the nature of toxicity profiles in animal species and humans, special studies addressing potential organ system toxicities may also be useful.

The CDER Division of Oncology Drug Products of the FDA welcomes discussion of specific anticancer drugs at the early stages of development to facilitate rapid and efficient drug development. Prior to filing an IND, sponsors may have discussions with appropriate FDA staff and request pre-IND evaluations of their study plan. This may help sponsors to avoid spending time and resources on unnecessary studies, and may help to expedite initiation of clinical studies of promising new drugs.

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