Severe and fatal toxicity in phase I human trials

What failures of preclinical prediction imply for investigational peptides

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Abstract

Preclinical activity and tolerability support the decision to investigate a treatment in humans, but their predictive value depends on the biological question and the model. This review examines severe or fatal toxicity during phase I development of TGN1412, BIA 10-2474, TAS266, MRX34 and affinity-enhanced MAGE-A3 T-cell receptor therapy. It also examines LTX-315 as a directly relevant short-peptide trial. The cases illustrate human immune-cell differences, unintended molecular recognition, incomplete representation of host responses, and the interpretation of animal findings. They are not interchangeable examples of uniformly negative animal toxicology. Some animal studies contained warnings; other apparently reassuring systems lacked the relevant human biology. LTX-315 establishes that a short peptide can produce severe clinical reactions, while the available reports do not establish that its animal safety programme missed a defined hazard. Trial phase, patient population, analysis denominator, severity and causal certainty are kept explicit. These selected cases support caution about using investigational peptides before adequate clinical evaluation; they cannot estimate the probability that any particular untested peptide will harm a person.

Scope and evidence approach

This is a focused narrative review of early human toxicity, rather than a review of late-stage efficacy failures. PubMed was searched with drug names, first-in-human and phase I terms, and terms for severe toxicity. A signed-in Consensus webpage was used in Deep mode to identify original clinical reports and trace experimental explanations. The NCBI Entrez and PMC literature skills were used to resolve publication records and available primary texts. Consensus summaries were treated as discovery aids; numerical statements and study conclusions were checked against original reports or original author abstracts.

The principal cases require a documented phase I human event and identifiable preclinical or mechanistic evidence. They are assessed for what the models actually showed, rather than being labelled collectively as proof that animal testing detected nothing. TAS266 and LTX-315 are included with explicit limits on the strength of the animal-prediction claim. The engineered T-cell example is a cell-therapy comparator, not a peptide drug.

Access was incomplete. Full texts were examined for several key clinical and mechanistic reports; other checks used the original authors' abstracts or identifiable full-text excerpts. The underlying GLP toxicology dossiers were not independently available for every programme. The accompanying evidence audit records these distinctions. Selection was purposive and provides neither an incidence estimate nor a comparison of the overall performance of animal and human-based methods.

"Severe" describes intensity; "serious" refers to outcomes such as death, life-threatening illness or hospitalization. The terms should not be substituted. In particular, a grade 3 laboratory abnormality is not automatically liver failure, and an event in a cancer patient is not automatically attributable to the investigational treatment. [17]

Clinical cases at a glance

Programme and population	Human observation	Interpretation of preclinical evidence
TGN1412; antibody; healthy volunteers	Six active recipients became critically ill; all survived. [1]	Strong experimental evidence of a human–macaque functional mismatch and inadequate assay presentation. [2,3]
BIA 10-2474; small molecule; healthy volunteers	Five of six active recipients in the 50 mg/day repeat-dose cohort had CNS serious adverse events; one died. [5]	Animal neurological findings existed. The clinical catastrophe was not adequately anticipated; the responsible molecular mechanism remains uncertain. [6,7]
TAS266; antibody-domain construct; advanced cancer	Three of four recipients developed grade 3 or worse liver-enzyme elevations at the starting dose. [8]	Preclinical potency and selected cell-safety findings did not resolve human safety; the original animal-model limitations were explicit. [9]
MRX34; liposomal RNA; advanced cancer	The daily-schedule report included 85 patients; four treatment-related deaths followed serious immune-associated events. [11]	Clinical authors report that animal GLP testing, including primates, did not predict the human immune-activation pattern. [10,11]
MAGE-A3 affinity-enhanced TCR T cells; cancer	The first two patients across two phase I protocols developed fatal cardiac toxicity. [12,13]	Human titin cross-recognition and a nonresponsive mouse counterpart explain a specific limitation of the models. [13]
LTX-315; short peptide; advanced cancer	Four of 39 patients had grade 3 hypersensitivity or anaphylaxis. [14]	Direct peptide evidence of severe human toxicity; these reports do not establish a defined animal false-negative. [14,15]

These denominators belong to different trials, populations, exposure schedules and outcomes. Their numerators must not be added to calculate a general risk of experimental drugs or peptides.

TGN1412: the relevant human immune cells were different

TGN1412 was a CD28 superagonist monoclonal antibody. In its first phase I trial, all six active-drug recipients developed systemic inflammatory symptoms within 90 minutes and critical illness within 12–16 hours, including pulmonary injury, renal failure and coagulation abnormalities. All survived after intensive care. The clinical outcome was therefore life-threatening toxicity, not six deaths. [1]

Experimental follow-up exposed a failure in the biological relevance of the preclinical systems. Stebbings and colleagues showed that presenting immobilized TGN1412 to human white blood cells produced marked cytokine release and proliferation; conventional assay presentation had been insufficiently informative. Their primate studies did not reproduce the same superagonist response. [2] Eastwood and colleagues then identified an important cellular difference: the human CD4

effector-memory population expressed CD28, whereas the corresponding macaque populations examined lacked it. [3]

The inference is more specific than a general statement that primates are poor models. An antibody can recognize the nominal target across species while the responsive cell population and resulting network behaviour differ. A reassuring dose in a species that lacks the relevant functional response provides limited reassurance about that response in humans. The experiments also show that merely replacing an animal test with an inadequately configured human-cell assay would not have solved the problem. [2,3]

BIA 10-2474: animal findings existed, but did not prevent fatal human toxicity

BIA 10-2474 was developed as an inhibitor of fatty acid amide hydrolase. The later original trial report documents five CNS serious adverse events among six active recipients receiving repeated 50 mg doses, including one death. [5] The earlier neurological report described only four consenting active recipients: three developed the acute syndrome and one remained asymptomatic. Its four-person subset should not replace the six-person treatment-cohort denominator. [4]

Calling this a case of “no toxicity in animals” would be inaccurate. The original cynomolgus toxicology paper describes tremor, weakness, incoordination and histological axonal changes at higher doses; it also describes early sacrifice or euthanasia in dose-finding or escalation. The authors judged certain findings reversible and assigned no-observed-adverse-effect levels. [6] Thus the question concerns the interpretation, relevance and management of observed findings as well as what was missed. The available studies do not isolate a single species difference as the cause of the clinical disaster.

Subsequent activity-based profiling demonstrated inhibition of additional lipid-processing enzymes and disruption of lipid metabolism in human neuronal cultures. These findings establish broader pharmacology than the intended target alone, but do not prove which interaction caused the patients' brain injury. [7] The clinical investigators likewise acknowledge that a complete mechanistic explanation remains unavailable. [5]

This case illustrates two distinct limits: initial human tolerability at lower exposures does not validate a later repeated-dose regimen, and a mechanistic explanation discovered after an event should not be described as established clinical causation merely because it is plausible.

TAS266: increased potency did not settle the safety question

TAS266 was a tetravalent antibody-domain construct designed to activate death receptor 5. In its first-in-human phase I study, three of four cancer patients developed treatment-attributed grade 3 or worse transaminase elevations at 3 mg/kg during the first cycle. The study stopped, and enzyme levels improved after discontinuation. Pre-existing TAS266-binding antibodies were found in the three affected patients. This was significant reversible biochemical hepatotoxicity; it should not be retold as fatal liver failure. [8]

The multivalent-nanobody experimental report demonstrated strong tumour-killing activity, including in mouse tumour models, and no greater toxicity to the cultured normal hepatocytes tested than comparator DR5 agonists. However, the authors explicitly recognized the limitations of non-crossreactive rodent systems, called for evaluation in a crossreactive species, and warned that anti-

drug responses might cause excessive cross-linking. [9] Publication of that discussion does not establish when each concern was available to the clinical decision-makers; it should not be presented as a documented pre-trial warning without further records.

The clinical authors proposed that potency, antibody-mediated clustering and possibly hepatocyte receptor expression contributed to toxicity, while stating that the mechanism was not fully elucidated. [8] This is therefore a useful case of efficacy and selected safety findings failing to resolve a clinically important risk. The accessible primary evidence does not support inventing a precise reassuring monkey safety margin or treating the hazard as wholly unforeseeable.

MRX34: toxicity emerged during expansion of a phase I programme

MRX34 delivered a microRNA-34a mimic in a liposomal formulation. The earlier phase I report included 47 patients on a twice-weekly schedule. [10] A later report described 85 patients treated on a daily-for-five-days schedule, with dose escalation and expansion. Five treatment-related serious events in expansion prompted closure; four were fatal. The later paper identifies an immune-associated pattern involving several organ systems. These were phase I events, but not all first-dose reactions or events in healthy volunteers. [11]

The clinical investigators describe animal GLP findings, including in nonhuman primates, involving platelets, complement and macrophage-related organ changes. That profile did not predict the human immune-activation syndrome. [11] These descriptions are the primary clinical authors' account of their animal programme; they do not constitute an independent reanalysis of all original animal data.

The responsible mechanism remains uncertain. The final investigators could not distinguish specific miRNA-mediated effects from nonspecific inflammatory responses to the RNA formulation or another explanation. They concluded that the serious immune-related risk outweighed the observed clinical activity and halted development. [11] This matters because “the molecule reached its biological target” and “the treatment is clinically acceptable” are separate propositions. Safety evaluation also continues after an initial dose-escalation cohort appears tolerable.

MAGE-A3 TCR therapy: a human cardiac peptide became an unintended target

Affinity-enhanced T cells were engineered to recognize an HLA-A1-presented MAGE-A3 tumour peptide. The first two treated patients, one with melanoma and one with myeloma, developed fatal cardiac toxicity in two phase I protocols. The clinical report documented severe myocardial damage and T-cell infiltration without detectable cardiac MAGE-A3 expression. [12]

Cameron and colleagues traced the response to a peptide derived from titin, an unrelated muscle protein. The engineered cells recognized human titin and killed differentiated, beating human cardiomyocytes. The equivalent mouse titin peptide did not activate the cells, leading the investigators to conclude that an HLA-A1 transgenic mouse would still have missed this particular specificity. [13] The same study reported the two deaths four and five days after administration and the reassessment of the first event after the second occurred. [13]

This example links a concrete molecular difference to a limitation of the animal model. It also demonstrates why mature tissue function can matter in human-cell testing. Its relevance to peptides

is conceptual: short sequences can have unanticipated biological recognition. The administered product here was genetically engineered cells; it was not an injected peptide supplement or a short-peptide drug.

LTX-315: direct short-peptide evidence, with a narrower prediction claim

LTX-315 is a nine-residue membranolytic peptide investigated by injection into tumours. In a phase I study of 39 patients, four had grade 3 hypersensitivity or anaphylaxis. The investigators nevertheless described an acceptable safety profile in the studied oncology setting; that judgment should be retained alongside the severe reactions, rather than replaced by a blanket claim of intolerability. [14]

Earlier original work in syngeneic mouse melanoma demonstrated local tumour destruction and protective immune responses. [15] These are efficacy and immunobiology observations. They do not, by themselves, establish that a matched animal safety experiment excluded the subsequent human reactions. The case belongs here because it provides direct evidence that a short peptide can cause severe clinical toxicity. It should not be presented as a proven species-specific failure without the necessary paired safety data.

There is also a difference between a monitored experimental anticancer treatment and using a different peptide for an unrelated purpose. Sequence length alone cannot transfer a safety assessment between products, routes, exposure schedules or patient populations. Neither the LTX-315 events nor the other selected cases provide a risk estimate for BPC-157 or another peptide marketed outside a clinical trial.

Implications for preclinical interpretation and peptide use

The cases support several connected conclusions. First, a model must represent the relevant target on the relevant cells and tissues. Second, pharmacological selectivity needs direct testing: a drug's intended target does not delimit every possible action. Third, biological activity can amplify through immune pathways or persist through an administered cell population. Fourth, the outcome of repeated exposure may differ from the outcome after an initial dose. These conclusions follow from the paired observations above; they do not imply that all mechanisms apply to all peptides.

Negative observations are conditional on the tested product, achieved exposure, route, duration, sample and measurements. A tumour-regression experiment answers a different question from a study designed to detect uncommon hypersensitivity. Even a biologically relevant system can miss a hazard when the susceptible host response or tissue state is absent. The uncertainty therefore has to be carried into the human development plan rather than converted into a categorical safety claim.

For clinical translation, the EMA first-in-human guideline emphasizes combining product quality, relevant nonclinical pharmacology, anticipated human biological activity and emerging human data. It addresses starting and maximum exposure, dose escalation, stopping rules and intervals between administrations and participants. These measures manage uncertainty; they do not guarantee its elimination. [16]

For an investigational peptide, the practical question is whether the actual formulation, route and intended use have adequate human evidence, including repeated-exposure safety and relevant follow-up. “It is a peptide,” “a related compound is approved,” “animals improved,” and “a few people reported no problem” do not supply that evidence. Phase I is an essential learning stage, and its

completion alone does not establish effectiveness or fully characterize rare and delayed harms. Clinical assessment must continue through the subsequent development programme and appropriate monitoring.

Boundaries of the review

Fialuridine is a consequential example of translational toxicity, but the fatal hepatitis B study was phase II. It is therefore not counted among the phase I cases here. [18] Later efficacy trials of other drugs address a different question and are not substitutes for the cases examined above.

The selected events cannot measure the frequency of preclinical failure, compare drug classes, or determine the risk of an individual experimental peptide. Oncology participants, healthy volunteers, antibody constructs, RNA formulations and engineered cells also differ materially. Some mechanistic explanations are experimentally well supported; others remain hypotheses. Preserving these distinctions makes the warning about premature human use stronger scientifically: important harm can remain unresolved despite substantial preclinical development, and unsupported reassurance should not stand in for clinical evidence.

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