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Safety, Toxicology, and Regulatory Science of CT Nano-Contrast Agents in Radiology

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Abstract

Nanoparticle-based contrast agents for X-ray computed tomography (CT) seek to overcome limitations of conventional iodinated small-molecule media but raise distinctive safety, toxicology, and regulatory science questions. Nano–bio-interactions specific to CT nanocontrast include reticuloendothelial system (RES) uptake, long-term hepatic and splenic retention, size-dependent renal versus hepatobiliary clearance, and potential immunogenicity and genotoxicity, all strongly influenced by particle composition, size, shape, surface charge, and coating chemistry. Robust preclinical evaluation requires harmonized in vitro assays, small, and large animal models, pharmacokinetic, and biodistribution studies designed according to emerging nanomaterial guidelines, and standardized reporting tailored to CT nano-agents. Regulatory frameworks from major agencies emphasize case-by-case assessment, enhanced physicochemical characterization, adapted toxicology programs, and post-marketing surveillance, while ethical and health-economic analyses must balance innovation against population-level safety, cost, and equity in access to nano-enabled CT. This review summarizes nano–bio-interactions most relevant to CT contrast safety, outlines a structured preclinical evaluation framework, and discusses regulatory, ethical, and economic considerations for deploying nanotechnology-based CT agents in routine diagnostics and screening.

Keywords: Nanoparticle-based contrast agents, X-ray computed tomography (CT), nano–bio- interactions, preclinical toxicology evaluation, regulatory science and safety assessment.

INTRODUCTION

Iodinated small-molecule contrast agents are the current standard for CT, but their rapid renal clearance, narrow imaging windows, and risk of contrast-induced kidney injury and hypersensitivity reactions are well-recognized limitations [1–5]. Nanoparticle-based CT contrast agents incorporating high-Z elements, such as gold, bismuth, tantalum, ytterbium, and cerium, have been developed to provide higher attenuation, prolonged circulation, and, in some cases, target-directed accumulation and theranostic capabilities [6–10]. These advantages are accompanied by altered biodistribution and persistence, including increased RES uptake, organ retention, and complex interactions with immune and hematologic systems [11–16].

Several reviews have described the design strategies and imaging performance of CT nanocontrast agents, yet systematic analyses of their safety, toxicology, and regulatory pathways remain less mature [17–23]. Regulatory agencies treat nanomedicines under existing medicinal product frameworks but expect enhanced characterization and adapted safety testing considering nano-specific behaviors [24–29]. There is, therefore, a need for a structured framework linking nano–bio-interactions, preclinical testing, regulatory expectations, and ethical and economic considerations specific to CT nano-agents.

This review first discusses the physicochemical and biological determinants of nano–bio-interactions relevant to CT contrast safety, including RES uptake, long-term organ retention, clearance pathways, immunogenicity, and genotoxicity. It then proposes a multi-tier preclinical evaluation framework covering

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in vitro assays, small, and large animal models, pharmacokinetics, and standardized reporting. Finally, it examines the evolving regulatory landscape, ethical issues, and health-economic considerations associated with deploying CT nanocontrast agents in routine diagnostic and screening pathways [30–37].

PHYSICOCHEMICAL BASIS OF NANO–BIO-INTERACTIONS IN CT NANOCONTRAST AGENTS

High-Z Cores and X-Ray Attenuation

CT contrast arises from differences in X-ray attenuation, which depends on tissue density, atomic number, and photon energy, with the photoelectric effect scaling strongly with atomic number [1, 15, 22]. High-Z elements, such as gold (Z = 79), bismuth (Z = 83), tantalum, and lanthanides, exhibit substantially higher mass attenuation coefficients than iodine at diagnostic CT energies, allowing significant contrast enhancement at lower molar concentrations when incorporated into nanoparticles [1, 4, 5, 15, 18, 20, 32]. Nanoparticulate formulations concentrate large numbers of high-Z atoms per particle, amplifying attenuation per binding or accumulation event in vivo [1–4, 15, 19, 20, 32, 38].

Core composition also influences biodegradability and potential toxicity: metallic gold is considered chemically inert but may persist in RES organs for months, whereas some bismuth compounds or oxides can partially dissolve or transform in physiological environments [5, 18, 20, 22, 28–31, 36]. Safe-by-design approaches for CT nanocontrast attempt to balance attenuation efficiency with predictable degradation and excretion profiles [31, 32, 35].

Size, Shape, Surface Charge, and Corona Formation

Size is a major determinant of pharmacokinetics and tissue distribution. Ultrasmall clusters (<5–6 nm hydrodynamic diameter) can pass through glomerular filtration and undergo rapid renal clearance, like conventional iodinated agents [23–25, 31, 35, 36]. Larger nanoparticles (10–100 nm) escape rapid filtration, display prolonged circulating half-lives, and preferentially accumulate in RES organs such as the liver and spleen [1–4, 15, 19, 20, 23–28, 32, 38, 39, 40]. Shape further modulates interactions: rods and plates exhibit different margination and cell uptake profiles compared with spheres, with gold nanorods showing anisotropic optical properties and distinctive cellular processing [6, 7, 13, 14, 23–28, 32].

Surface charge and coating chemistry control colloidal stability, opsonization, complement activation, and protein corona composition [1–4, 23–28, 31–36, 40]. Neutral or slightly negative, PEGylated surfaces usually reduce plasma protein adsorption and RES uptake, whereas strongly positive surfaces tend to interact more with cell membranes and plasma proteins, potentially increasing toxicity [1–4, 23–28, 31–36]. Protein coronas formed in vivo alter the effective "biological identity" of nanoparticles, affecting immune recognition and biodistribution in ways not always predictable from in vitro measurements [23–28, 36].

Passive and Active Targeting in Relation to Safety

Many CT nanocontrast agents exploit the enhanced permeability and retention (EPR) effect in tumors and inflamed tissues, where leaky vasculature and poor lymphatic drainage permit nanoparticle extravasation and retention. [16, 17, 32] Others incorporate targeting ligands – peptides, antibodies, aptamers – that bind tumor or stromal receptors to enhance local concentration [1–4, 6, 7, 10, 11, 24, 32, 40]. While these strategies can improve imaging performance, they also modify safety profiles by changing tissue distribution, cellular uptake, and clearance dynamics [1–4, 10, 11, 16, 17, 24, 32, 37–39, 41].

In preclinical models, EPR often appears strong, but in human tumors, it is heterogeneous and sometimes weak, which affects both efficacy and exposure of normal tissues [16, 17, 32, 38]. Active targeting may enhance uptake in receptor-positive compartments while increasing exposure of unintended tissues expressing the same receptors, raising new off-target toxicity concerns [4, 10, 11, 16, 17, 24, 32, 38].

NANO–BIO-INTERACTIONS: RES UPTAKE, CLEARANCE, IMMUNOGENICITY, GENOTOXICITY

Nano-enabled CT contrast agents interact with biological systems in ways that are qualitatively and quantitatively different from conventional iodinated molecules [1–5, 18, 20, 23–28, 31–36, 38, 39, 40].

Key processes include reticuloendothelial system (RES) uptake and long-term organ retention, size-dependent renal versus hepatobiliary clearance, immunotoxicity, and complement activation, and potential genotoxic and carcinogenic effects mediated by oxidative stress or chronic inflammation [16, 17, 23–28, 31–36, 38, 39]. Understanding these nano–bio-interactions is essential for designing safe CT nano-agents and for constructing preclinical testing strategies that satisfy regulatory expectations.

RES Uptake and Long-Term Organ Retention

Mechanisms and Kinetics of RES Uptake

Following intravenous injection, nanoparticles circulate through the vascular system and encounter RES cells predominantly in the liver, spleen, and bone marrow [1–5, 18, 20, 23–28, 31–36, 38, 39, 40]. Kupffer cells in liver sinusoids and splenic macrophages recognize and internalize particles via opsonin-mediated phagocytosis, scavenger receptors, and pattern-recognition receptors [23–28, 31–36] This process is strongly influenced by the protein corona, which can be enriched in complement components, immunoglobulins, and other opsonins depending on nanoparticle surface chemistry [23–28, 31–36].

Studies of gold nanoparticles used as CT contrast agents have shown that a large fraction of the injected dose can accumulate in the liver within minutes to hours, often with a size- and shape-dependent pattern [19, 21, 23–28, 30, 33, 34, 38, 39, 40]. Small spherical particles remain in the bloodstream longer and distribute more widely before eventual RES uptake, whereas larger spheres and nanorods tend to deposit rapidly in the liver and spleen [23–28, 30, 33, 34, 38, 39, 40]. High-resolution imaging of liver sections has documented the rapid association of gold nanoparticles with sinusoidal endothelial cells and their subsequent transfer to Kupffer cells, with long-lasting localization in lysosomal compartments [23, 24, 27, 28, 33, 34, 40].

Bismuth-based nanoparticles and other high-Z systems show broadly similar behavior, although their rates of uptake and subsequent processing depend on core chemistry and coating [5, 18, 22, 28–31, 36, 38, 39]. For example, Bi, and S nanorods coated with hydrophilic polymers display robust liver enhancement on CT and strong microscopically visible accumulation in Kupffer cells, while some bismuth chelates distribute and clear more like small molecules [5, 22, 28–31, 36].

Long-Term Retention and Tissue Remodeling

Because many metallic cores are not readily biodegradable, RES uptake often leads to prolonged retention [23–28, 31–36, 40]. In a systematic study of spherical gold nanoparticles of different sizes and coatings, long-term retention in liver was observed over at least 47 days, with particles remaining largely intact inside lysosomes regardless of geometry or surface functionalization [33]. Another study of BSA-coated 20 nm gold nanoparticles demonstrated that substantial fractions of the injected dose remained in liver, spleen, and kidney 120 days after a single intravenous administration, with only partial reduction in liver burden and paradoxical increases in splenic and renal burdens over time [23, 27].

Histological evaluation in that long-term study revealed early inflammatory cell infiltration and fibrotic changes in liver and spleen, and more pronounced inflammatory and fibrotic responses in kidneys despite relatively low gold levels in renal tissues, suggesting systemic or indirect mechanisms of kidney injury [23, 27]. By contrast, another long-term retention study noted that although gold nanoparticles remained in liver endothelial cells and Kupffer cells for weeks, conventional liver enzyme tests did not show overt toxicity, underscoring that biochemical markers can underestimate subtle structural or functional changes [33].

Similar concerns apply to other high-Z systems. Bismuth nanoparticles and polyoxotungstate clusters can accumulate in liver and spleen, and while some studies report minimal short-term toxicity at imaging-relevant doses, long-term effects remain understudied [5, 18, 22, 28–31, 36]. For nanoceria, the balance between ROS-scavenging and potential pro-oxidant behavior over long-time frames is still being clarified [35, 36, 42].

Collectively, these data indicate that even when CT nano-agents appear clinically safe in the short term, their slow tissue elimination and long-term biopersistence warrant careful chronic toxicity and histopathology studies, especially in organs showing high uptake or cumulative exposure [23–28, 31–36, 38, 39, 40].

Renal Versus Hepatobiliary Clearance and Kidney Risk

Size-Dependent Clearance Pathways

Clearance route is highly size-dependent. Ultrasmall clusters and nano-constructs with hydrodynamic diameters below the glomerular filtration threshold can be eliminated rapidly via urine, reducing long-term RES load but potentially exposing renal tissue to higher transient concentrations [23–27, 31, 34–36, 38, 39, 40, 43]. In vivo studies with glutathione-protected gold nanoclusters have shown efficient renal clearance and low systemic toxicity, whereas larger BSA-protected clusters formed aggregates, leading to increased liver and spleen accumulation and potentially irreversible toxic responses [26].

Systematic evaluations of size-dependent toxicity and biodistribution over periods extending to 90 days have revealed that mid-sized gold nanoparticles (tens of nanometers) can produce more pronounced organ accumulation and histological changes than both ultrasmall clusters and larger particles, due to a combination of prolonged circulation and efficient RES uptake [23–28, 31–36, 38, 39, 40]. These findings suggest that fine-tuning size around the renal filtration threshold is critical to balance rapid clearance and organ exposure.

Renal Safety and Comparison with Iodinated Agents

Conventional iodinated contrast media are well known for their nephrotoxic potential in susceptible patients, and renal safety is a central driver for interest in nano-CT agents [2, 4, 19, 20, 32, 40, 43]. Animal studies comparing gold nanoparticle contrast with iodinated media have reported that gold nanoparticles can achieve equal or superior contrast enhancement at lower radiation doses and may reduce contrast-induced nephropathy risk in certain models, although human data remain sparse [2, 4, 19, 20, 32].

Ultrasmall renal-clearable CT nano-agents, such as certain gold clusters or tungsten-based complexes, exhibit rapid urinary excretion and relatively low renal histopathology at imaging doses, but some studies have noted subtle tubular changes or biomarker elevations at high or repeated doses [23–27, 31, 34–36, 38, 40]. Regulatory evaluation must, therefore, consider not only absolute nephrotoxicity risk but also whether nano-agents allow lower total contrast loads, reduced radiation doses owing to higher attenuation, or improved diagnostic accuracy that offsets residual renal risk [2, 4, 19, 20, 38, 39, 40, 43].

For larger, hepatobiliary-cleared nano-agents, kidney risks may be lower at equivalent doses, but they are not eliminated, and careful renal monitoring remains advisable, especially in patients with pre-existing kidney disease or cumulative exposures [5, 18, 22, 23–28, 31, 34–36, 38, 39, 40].

Immunotoxicity, Complement Activation, and CARPA

Mechanisms of Nanoparticle-Induced Complement Activation

Nano-contrast agents can activate complement through multiple pathways – classical, lectin, and alternative – via surface charge, pattern recognition, or corona-mediated interactions with complement proteins [23–28, 31–36, 40]. Complement activation results in the generation of anaphylatoxins (C3a, C5a) and the terminal complement complex (C5b-9), which can trigger vasodilation, increased vascular permeability, smooth muscle contraction, and leukocyte activation.

In the clinic, such complement activation can manifest as complement activation-related pseudoallergy (CARPA), a spectrum of infusion reactions that mimic anaphylaxis but are not mediated by IgE. CARPA has been well documented for several liposomal drugs and intravenous nanoparticle formulations; its incidence for CT nano-agents remains uncertain because few have advanced to clinical trials.

Factors that increase CARPA risk include:

- Highly charged or hydrophobic surfaces.
- Dense protein coronas enriched in complement-activating patterns.
- High bolus doses or rapid injection rates.
- Pre-existing complement dysregulation or prior sensitization [23–28, 31–36, 40].

Assessment and Mitigation of Immunotoxicity

Nonclinical immunotoxicity testing for nano-agents typically follows a tiered approach [37–39, 41, 44–45].

- *In-vitro complement assays* using human serum or plasma quantify C3a, C5a, and sC5b-9 formation and can reveal which pathways are engaged. [23–28, 31–36, 40].
- *Cytokine release assays* with human PBMCs or whole blood provide information on potential cytokine storms or pro-inflammatory responses. [23–28, 31–36, 40].
- *Hemocompatibility panels* assess hemolysis, coagulation, platelet activation, and leukocyte activation. [23–28, 31–36, 40].
- *In-vivo CARPA models*, typically in pigs or dogs, evaluate hemodynamic changes and complement activation under clinically relevant infusion conditions.

Mitigation strategies include optimization of surface chemistry (e.g., hydrophilic coatings, reduced charge), careful control of particle size and aggregation, and clinical measures, such as slower infusion rates and premedication with antihistamines or corticosteroids [23–28, 31–36, 40]. Some experimental approaches involve complement inhibitors like modified factor H constructs to prevent excessive complement activation in high-risk settings, though their use in CT imaging is not yet established.

Genotoxicity, Oxidative Stress, and Carcinogenicity

Mechanisms of Nanoparticle-Related Genotoxicity

Nanoparticles can induce genotoxicity through direct and indirect mechanisms [26, 34, 36, 40]. Direct mechanisms include physical interactions with DNA or chromosomal structures if particles enter the nucleus, which may be more relevant for ultrasmall or flexible structures [26, 34, 36, 40]. Indirect mechanisms are often more prominent and involve:

- Generation of reactive oxygen and nitrogen species.
- Activation of inflammatory pathways leads to bystander DNA damage.
- Release of toxic ions or degradation products.
- Interference with DNA repair processes [26, 34, 36, 40].

Diagnostic CT doses alone are not sufficient to cause significant genotoxic risk in most patients, but localized high concentrations of high-Z nanoparticles could potentially amplify radiation-induced DNA damage in specific microenvironments [10, 11, 15, 20, 35, 40]. While this is a desirable effect in radiosensitizing therapeutic contexts, it requires careful evaluation in purely diagnostic applications, especially in radiosensitive populations such as children.

Adapting OECD Genotoxicity Test Guidelines to Nanomaterials

Traditional OECD Test Guidelines for genotoxicity (e.g., TG 476, 487, and 473) were developed for soluble chemicals and present challenges when applied to nanomaterials [41]. Issues include sedimentation of particles in culture wells, limited cellular uptake, interference with assay readouts, and lack of chronic exposure representation [36]. In response, the OECD has developed guidance on adapting in vitro micronucleus tests and other assays to better accommodate nanomaterials, including recommendations on:

- Characterizing nanoparticle dispersion and stability in test media.
- Adjusting exposure times and concentrations.
- Using appropriate positive and negative controls for nanomaterials.
- Combining in-vitro data with in-vivo genotoxicity and toxicokinetic information [41].

For CT nano-contrast agents, regulators will expect at least a basic genotoxicity battery adapted for nano-specific considerations (Table1), particularly for redox-active agents, slowly cleared, or likely to be used in repeated examinations over many years [36, 41].

Table 1. Key nano–bio-interactions relevant to CT nanocontrast safety.

Interaction domain	Main determinants	Typical findings for CT nanocontrast agents	Safety implications
RES uptake and retention [1–5, 18, 20, 23–28, 32–36, 45]	Size >10–15 nm, opsonization, core biodegradability	High liver/spleen accumulation; long-term retention of Au/Bi systems; Kupffer-cell storage	Need for chronic toxicity, fibrosis assessment, accumulation modelling, especially for repeat dosing.
Renal vs hepatobiliary clearance [5, 18, 20, 23–27, 31, 34–36 ,39, 40]	Hydrodynamic size, surface charge, solubility of degradation products	Ultrasmall clusters: fast renal clearance; larger particles: RES uptake and biliary excretion	Trade-off between reduced long-term retention and nephrotoxicity vs increased organ storage but less glomerular stress.
Immunogenicity and complement [23–28, 33, 34, 36, 45]	Surface chemistry, corona, ligand density, formulation impurities	Complement activation and cytokine release in some systems; others show minimal effects	Necessitates CARPA testing, infusion-rate control, and immunotoxicity panels; informs clinical monitoring.
Genotoxicity and oxidative stress [26, 34, 36, 45]	Redox activity, ion release, chronic inflammatory milieu	Mostly low genotoxicity at clinical doses, but DNA damage at high or repeated exposures in some models	Requires adapted in-vitro and in-vivo genotoxicity and mechanistic studies for redox-active cores.
Hemodynamic and microvascular effects [23–28, 31–36, 45]	Injection rate, viscosity, aggregation	Transient hemodynamic changes reported; potential microvascular plugging at very high doses	Cardiovascular safety pharmacology and safe infusion protocols must be defined before clinical use

FRAMEWORK FOR PRECLINICAL SAFETY EVALUATION OF CT NANOCONTRAST AGENTS

Regulatory Context and Overarching Principles

Regulators do not define nanomedicines as a separate legal category but expect "enhanced" evaluation where nano-scale properties alter quality, safety, or efficacy [38–39, 42–45]. For CT nanocontrast agents, this implies comprehensive physicochemical characterization, adapted toxicology, detailed pharmacokinetics and biodistribution, and justification of clinical starting doses using the totality of evidence [15, 31–33, 37, 38–41].

Position papers and horizon-scanning reports emphasize case-by-case assessment, with particular concern for unusual biodistribution, persistence, and immunological responses [38–39, 42–45]. A credible preclinical package integrates in vitro mechanistic work, rodent pharmacology and toxicology, and large animal data addressing hemodynamics, immunotoxicity, and imaging performance [1–5, 15, 19–22, 31–33, 37–39, 41].

Physicochemical Characterization and Reference Standards

Physicochemical characterization for CT nanocontrast agents should cover:

- Core composition and crystallinity (e.g., XRD, ICP-OES).
- Size and size distribution (TEM, DLS, NTA).
- Shape (TEM, SEM).
- Surface charge (zeta potential).
- Coating chemistry and grafting density (XPS, NMR, elemental analysis).
- Colloidal stability in buffer, serum, and clinically relevant media [1–4, 18, 20, 23–28, 31–36, 40].

Assessments before and after incubation in biological fluids help capture protein corona effects and aggregation tendencies [23–28, 31–36, 45]. Use of reference nanomaterials and inter-laboratory comparisons enhances confidence in measurements and can facilitate regulatory review [39, 41].

In-Vitro Testing Strategy

In vitro assays provide early insight into cytotoxicity, hemocompatibility, immunotoxicity, and genotoxicity but must be designed to avoid artefacts (e.g., sedimentation, adsorption of assay reagents). [26, 34, 36] For CT nanocontrast agents, a practical battery includes:

- Cytotoxicity in renal (e.g., HK-2), hepatic (e.g., HepG2), endothelial, and macrophage cell lines, using multiple orthogonal readouts (MTT/XTT, LDH release, live–dead staining) [23–28, 31–36, 39].
- *Hemocompatibility:* hemolysis, coagulation tests (PT, APTT), platelet aggregation, and leukocyte activation assays. [23–28, 31–36, 39].
- Complement activation and cytokine release assays in human serum/plasma or whole blood [23–28, 33, 34, 36, 40].
- Genotoxic screens (micronucleus, comet assay) adapted for nanoparticles [26, 34, 36–45].

These data inform dose selection and safety margins for in vivo work and can identify problematic formulations early [37, 41].

Small animal Pharmacokinetics, Biodistribution, and Toxicology

Rodent studies evaluate pharmacokinetics (PK), biodistribution (BD), acute and subchronic toxicity, and preliminary imaging performance. [1–5, 15, 19–28, 31–36, 38–40, 42–45]. Key design features include:

- Intravenous administration at multiple dose levels, including anticipated clinical exposure and multiples thereof [37, 40, 41].
- Serial blood sampling for PK analysis (clearance, volume of distribution) [23–27, 31, 34, 37, 41].
- Quantitative organ concentration measurement (e.g., ICP-MS) at multiple time points, with corresponding histopathology and clinical chemistry [23–28, 31–36, 45].
- In vivo CT imaging to relate enhancement curves to tissue concentrations and validate imaging–PK relationships [1–5, 15, 19–22, 31–33, 38, 39].

Guidelines for nanomaterial PK study design emphasize adequate sampling windows, species selection, and control of confounders to produce interpretable and regulatory-acceptable data [37, 41].

Large Animal Models and Translational end Points

Large animal models (e.g., pigs, dogs, non-human primates) are particularly important for CT nanocontrast agents to evaluate:

- Hemodynamic responses and CARPA risk under clinically relevant infusion rates and volumes.
- Renal safety in a kidney physiology closer to humans (Table 2).
- Imaging performance in human-scale anatomies and scanner hardware [19–22, 38–39].

These data support the design of first-in-human studies by informing maximum tolerated dose, infusion protocols, monitoring requirements, and dose–response relationships for CT enhancement [38, 39, 40].

Standardized Reporting and Study Quality

High-quality, transparent reporting facilitates regulatory review and cross-study comparison [1–5, 15, 19–22, 31–33, 37–39, 41–43]. Recommended elements include:

- Full nanoparticle characterization, including batch-to-batch variability.
- Complete description of animal models, randomization, blinding, and power calculations.
- Clear dose metrics (mg/kg, mg element/kg, particle number).
- Detailed time-course data for PK, BD, and organ toxicity.
- Adherence to ARRIVE and nanomedicine-specific extensions [37, 40–41].

IMMUNOTOXICITY ASSESSMENT FOR CT NANO-CONTRAST AGENTS

Appropriate immunotoxicity assessment is central to the development of intravenous nano-CT agents (Table 3). A structured toolbox can be deployed from in vitro screening to in vivo confirmation.

Table 2. Core components of a preclinical safety package for CT nanocontrast agents.

Level	Core components	Example outputs relevant to CT nanocontrast safety
Physicochemical characterization [1–5, 18, 20, 23–28, 31–36, 44]	Size, shape, composition, surface chemistry, stability, corona	TEM/DLS size distributions; zeta potential; XRD; XPS; serum stability; corona proteomics.
In-vitro assays [23–28, 31–36]	Cytotoxicity, hemocompatibility, complement activation, cytokine release, genotoxicity	IC in key cell lines; hemolysis %; coagulation/platelet effects; complement split products; cytokine profiles; micronucleus data.
Rodent PK/toxicology [1–5, 15, 19–22, 23–28, 31–36, 39, 40]	PK/BD, acute/subchronic toxicity, histology, imaging	t , CL, Vd; organ concentrations vs time; liver/kidney histopathology; CT enhancement vs concentration curves.
Large animal studies [19–22, 39, 40–45]	Hemodynamic stability, CARPA risk, renal safety, clinical-scale imaging	Blood pressure, heart rate, pulmonary pressures; complement markers; renal biomarkers; human-scale CT images and HU–time curves.
Integrated safety assessment [37–45]	Weight-of-evidence analysis, MABEL selection, risk management plan	First-in-human starting dose justification; contraindications; monitoring protocols; risk minimization measures.

Table 3. Immunotoxicity assessment toolbox for CT nano-contrast agents.

Level / assay type	Example methods	Main readouts	Relevance for CT nanocontrast safety
In-vitro complement activation [23–28, 33, 34, 36, 45]	Human serum/plasma: ELISA for C3a, C5a, sC5b-9; CH50/AH50; complement deposition assays	Degree/pathway of complement activation; CARPA risk estimation	First-line screen for pseudoallergy; helps prioritize formulations and infusion-rate limits.
Cytokine release assays [23–28, 33, 34, 36, 40]	Human PBMCs or whole blood; multiplex cytokine panels	Cytokine storm potential; pro-inflammatory vs tolerogenic profiles	Predicts acute infusion reactions and interactions with immunotherapies.
Hemocompatibility [23–28, 33, 34, 36]	Hemolysis, coagulation panels, platelet/leukocyte activation	RBC lysis; coagulopathy; platelet and leukocyte activation	Essential for IV agents; guides safe clinical dosing and contraindications.
In-vivo CARPA models [1–13]	Pig/dog models with invasive hemodynamic monitoring	Blood pressure, heart rate, pulmonary artery pressure, complement markers	Approximates clinical infusion reactions; informs clinical protocols and premedication.
Adaptive immune response [26, 36–45]	Anti-particle and anti-PEG antibodies; T-cell responses	Antibody formation; accelerated clearance; autoimmunity potential	Important for repeated dosing and screening applications.
Immunosuppression / immunostimulation [26, 36, 40]	NK cell function; T-cell proliferation; macrophage assays	Changes in innate/adaptive function; infection and autoimmunity risk	Supports labeling for immunocompromised patients and those on immunomodulators.

REGULATORY, ETHICAL, AND HEALTH-ECONOMIC CONSIDERATIONS

Regulatory Landscape and Classification

Agencies, such as the EMA and FDA, classify nano-enabled CT agents under existing medicinal product frameworks while explicitly recognizing nano-specific issues [38–40, 42–45]. Nanocontrast agents may be regulated as drugs, devices, or combination products depending on composition, mechanism, and intended use, and classification affects the primary review path and data requirements (Table 4) [38–40, 42–45].

Horizon-scanning reports highlight nanotechnology-based medicinal products as emerging priorities and emphasize the need for regulatory science investment [40].

Adapting ICH and OECD Guidance

Current ICH quality and safety guidelines are adaptable to nanomedicines but often require explicit nano-focused interpretation [37, 41, 40]. Examples include considering particle size distribution as a

critical quality attribute in ICH Q8/Q11 terms or extending repeat-dose toxicology durations to capture slow clearance in ICH S-guideline contexts [37, 38, 45]. OECD test guidelines are being updated to account for nano-specific issues, particularly in genotoxicity and ecotoxicology (Table 5) [39, 41].

Table 4. Regulatory and ethical issues specific to CT nano-contrast agents.

Domain	Key questions	Implications
Regulatory classification [38–40, 42–45]	Drug, device, or combination product? Theragnostic?	Shapes primary review pathway, required studies, and post-marketing obligations.
Nano-specific safety expectations [37, 40–41]	What additional tests are beyond conventional contrast agents?	Enhanced characterization; adapted tox and immunotoxicity; chronic retention assessment.
Risk–benefit balance [2, 4, 19, 20, 38–40]	Do benefits clearly outweigh uncertain long-term risks?	Influences indication selection (high-risk vs screening), trial design, labeling, and risk management.
Informed consent and communication [5–13]	How to explain nano-specific uncertainties to patients?	Requires clear, non-sensational patient materials detailing potential retention and unknowns.
Equity and access [38–39, 44]	Will high-cost nano-contrast widen disparities?	May require pricing, reimbursement, and policy measures if nano-CT becomes standard in some cancers.

Table 5. Comparative safety profile: Iodinated small-molecule vs nano-based CT contrast agents.

Dimension	Iodinated small-molecule agents [2, 4, 19, 20, 32, 43]	Gold-based nano-CT agents [1, 3, 4, 15, 19–21, 23–28, 30, 33, 34, 39]	Other nano-CT agents: Bi, composites, nanoceria [1, 3–5, 11, 15, 18, 19, 20, 22, 28–31, 32, 35, 36, 40]
Core chemistry	Tri-iodinated organic molecules	Elemental Au nanoparticles with polymer/protein coatings	BiS Bi chelates, lanthanides, I–Au composites, metal oxides, nanoceria.
Size	Molecular	2–100 nm	2–150 nm.
Clearance	Renal, rapid	Size-dependent; renal for clusters, RES/biliary for larger	Chelates renal; particulate systems RES/biliary.
Imaging window	Seconds–minutes	Prolonged (hours)	Intermediate to prolonged.
Acute toxicity	CIN, osmotic/viscosity effects, allergy	Low acute toxicity at imaging doses; CARPA risk not fully defined	Generally low acute toxicity: hydrolytic instability may increase risk.
Long-term retention	Minimal	Documented RES retention with partial clearance	RES persistence variably documented; long-term fate less characterized.
Nephrotoxicity	Well established	Potential with clusters; mitigated with larger particles but shifted organ risk	Chelates like iodine; particulate forms may reduce nephrotoxicity but raise hepatosplenic concerns.
Immunogenicity	Hypersensitivity known	Complement activation possible; immune profiling needed	Similar concerns; limited clinical data.
Evidence base	Extensive	Mainly preclinical; few human studies	Predominantly preclinical; early clinical tumor studies emerging.

Health-Economic and Implementation Aspects

Introducing nano-CT agents will involve higher acquisition costs and potential changes in workflow, which must be justified by clinical benefits and reduced adverse events [2, 4, 19, 20, 38–40]. Early use cases are likely in high-value niches: oncology, complex vascular imaging, and patients at high risk for nephrotoxicity or hypersensitivity (Table 6) [1, 3–5, 8, 11, 18–22, 28–32, 35, 38–40].

Table 6. Health-economic and implementation considerations: Conventional vs nano-CT contrast.

Dimension	Iodinated CT contrast [2, 4, 19, 20, 32, 40, 43]	Nano-CT contrast agents [1, 3–5, 8, 11, 15, 18–22, 28–32, 35, 38–40, 43]	Implications
Acquisition cost	Low; generic	High; complex manufacturing	Strong evidence of benefit needed to justify reimbursement.
Infrastructure	Widely available	May require dual-energy CT, specific protocols	Adoption initially at tertiary centers.
Benefit profile	Effective; limited molecular specificity; nephrotoxicity/allergy	Enhanced contrast, longer windows, potential targeting; retention uncertainties	Niche use in high-risk/high-benefit settings first.
Adverse events	Well characterized	Uncertain long-term retention/immunology	Registries and post-marketing studies critical.
Workflow	Short windows; precise timing	Longer windows; potential exam re-timing	Requires radiology workflow redesign in some settings.
Equity	Broad access	Risk of availability only in high-resource centers	Policy interventions are needed if standard of care shifts.

CONCLUSIONS AND FUTURE DIRECTIONS

CT nanocontrast agents based on high-Z nanoparticles offer compelling advantages in attenuation efficiency, imaging window length, and potential molecular targeting, but they introduce novel safety, toxicology, and regulatory challenges [1–5, 11, 15, 18–22, 28–32, 35, 38–40, 43]. Nano–bio- interactions, such as RES uptake, long-term organ retention, size-dependent clearance, immunogenicity, and possible genotoxicity, must be carefully characterized and managed through a structured preclinical program [1–5, 18, 20, 23–28, 31–36, 40].

Regulatory science is evolving toward clearer expectations for nanomedicines, stressing enhanced characterization, adapted toxicology, and robust post-marketing surveillance, while ethical and health-economic considerations demand transparent communication and equitable access strategies [37–45]. As evidence accumulates from well-designed preclinical and clinical studies, CT nano-agents may move from specialized research tools to clinically accepted options in carefully selected indications, provided that safety and value are convincingly demonstrated [1–5, 15, 19, 20, 32, 37–41, 43].

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