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1 Biodistribution and targeting properties of iron oxide
2 nanoparticles for treatments of cancer and iron anemia
3 disease

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23 **ABSTRACT:** IONP commercialized for treatments of iron anemia or cancer diseases can be
24 administered at doses exceeding 1 gram per patient, indicating their bio-compatibility when they are
25 prepared in the right conditions. Various parameters influence IONP biodistribution such as
26 nanoparticle size, hydrophobicity/hydrophilicity, surface charge, core composition, coating properties,
27 route of administration, quantity administered, and opsonization. IONP biodistribution trends include
28 their capture by the reticuloendothelial system (RES), accumulation in liver and spleen, leading to
29 nanoparticle degradation by macrophages and liver Kupffer cells, possibly followed by excretion in
30 feces. To result in efficient tumor treatment, IONP need to reach the tumor in a sufficiently large
31 quantity, using: i) passive targeting, *i.e.* the extravasation of IONP through the blood vessel irrigating
32 the tumor, ii) molecular targeting achieved by a ligand bound to IONP specifically recognizing a cell
33 receptor, and iii) magnetic targeting in which a magnetic field gradient guides IONP towards the tumor.
34 As a whole, targeting efficacy is relatively similar for different targeting, yielding a percentage of
35 injected IONP in the tumor of $5 \cdot 10^{-4}$ to 3%, 0.1 to 7%, and $5 \cdot 10^{-3}$ to 2.6% for passive, molecular, and
36 magnetic targeting, respectively. For the treatment of iron anemia disease, IONP are captured by the
37 RES, and dissolved into free iron, which is then made available for the organism. For the treatment of
38 cancer, IONP either deliver chemotherapeutic drugs to tumors, produce localized heat under the
39 application of an alternating magnetic field or a laser, or activate in a controlled manner a sono-
40 sensitizer following ultrasound treatment.

41

42 **KEYWORDS:** iron oxide nanoparticle, toxicity, pharmacokinetic, biodistribution, liver toxicity, kidney
43 toxicity, iron anemia.

44 **ABBREVIATIONS:**

45 Admin. Route: Route of administration.

46 BBB: Selective semi-permeable membrane barrier that separates the circulating blood from the brain
47 and extracellular fluid in the central nervous system.

48 CKD: Chronic kidney disease.

49 CE marked: CE marked medical devices, such as those containing nanoparticles, are allowed for
50 commercialization in Europe.

51 CT: circulation time, i.e. the time required for IONP to flow between two given points;.

52 $t_{1/2}$: blood half-life, *i.e.* the time it takes for IONP to have their concentration decreased by a factor of 2
53 following their administration.

54 CTX: Cyclophosphamide.

55 DMSA: Dimercaptosuccinic acid

56 EMA: European Medicines Agency.

57 EPR: Enhanced permeability and retention effect is a mechanism enabling IONP to accumulate in tumor
58 tissue more than in normal tissues.

59 FDA: Food and drug agency in USA.

60 IONP: Iron oxide nanoparticles, composed of a maghemite or magnetite core surrounded by a coating
61 material, displaying superparamagnetic or ferrimagnetic magnetic behaviors, of sizes 1 to 100 nm.

62 iv: intravenous.

63 MT: Magnetic targeting or application of a magnetic field gradient on IONP to target the tumor with
64 IONP.

65 MDT: Molecular drug targeting used to target a tumor.

66 MTO: Mitoxantrone.

67 MTX: Methotrexate.

68 MRI: Magnetic resonance imaging.

69 MW: Molecular weight.

- 70 PVA: Polyvinyl alcohol.
- 71 %ID: Percentage of the injected dose that ends up in the tumor.
- 72 Quantity admin.: Quantity administered.
- 73 RES: Reticuloendothelial system is a network of cells and tissues, in blood, general connective tissue,
74 spleen, liver, lungs, bone marrow, and lymph nodes.
- 75 SPIO: Superparamagnetic iron oxide nanoparticles having a thermally unstable magnetic moment.
- 76 TA: Targeting agent used to target a tumor.
- 77

78 INTRODUCTION

79 In the medical field, IONP (iron oxide nanoparticles) have attracted much attention due to several of
80 their appealing properties such as: i) their faculty to efficiently release free iron in the organism and
81 fight against iron anemia disease, (Auerbach2017), ii) the coupling of their magnetic moment with an
82 external magnetic field that improves the quality of the contrast in magnetic resonance imaging (MRI),
83 (DiMarco2007), can yield efficient magnetic drug targeting (MDT), (Janko2013), or produce localized
84 heat in magnetic hyperthermia, (Perigo2015), iii) the absorption of laser light resulting in efficient
85 photothermal therapy, (Estelrich2018), iv) the binding of targeting agents, chemotherapeutic drugs, or
86 sonosentizers, which can increase the quantity of IONP reaching the tumor and/or enhance anti-tumor
87 activity, (Gobbo2015). Figure 1 summarizes these various medical applications of IONP. Although
88 belonging to a specific category of nano-product, IONP are characterized by a series of different
89 physico-chemical properties: i) amorphous or crystallized structures, (Phu2011), ii), multiple iron oxide
90 compositions and crystallographic structures, such as magnetite (Fe_3O_4) or maghemite (Fe_2O_3),
91 (Salazar2011), iii), different sizes, size distributions, and hydrodynamic diameter, typically comprised
92 between 1 and 100-500 nm, (Wu2008), iv), various shapes or geometries including isotropic ones such
93 as cubic and spherical, (Zhen2011), and elongated ones such as elliptical, (Freitas2015), v), various
94 surface charges, typically comprised between -40 and 30 mV, (Sakukhua2015), vi), magnetic properties
95 most commonly leading to superparamagnetism or ferrimagnetism with unstable or stable magnetic
96 moment, respectively, (Wu2015), vi), the presence of various coating materials, targeting agents,
97 sonosensitizers, and/or chemotherapeutic drugs surrounding the iron oxide core, (Laurent2008). In
98 general, it is possible to tune these properties to adjust IONP biodistribution and activity in the
99 organism, making these nanoparticles an excellent system to foresee efficient treatments of cancer and
100 iron anemia disease. Here, various IONP fabrication methods as well as the main properties of
101 commercialized or CE marked IONP preparations are first described. Second, IONP biodistribution
102 properties as well as the main parameters influencing them are presented. Third, the different types of
103 targeting strategies that enable IONP to reach the tumor in sufficiently large quantity to trigger anti-

104 tumor activity are highlighted. They include passive targeting through the EPR (Enhanced permeability
105 and retention) effect as well as molecular and magnetic active targeting. Fourth, the mechanisms of
106 actions are explained. They involve the capture of IONP by the RES (reticuloendothelial system)
107 followed by the dissolution of IONP into free iron for the treatment of iron anemia disease. In cancer
108 treatment, they are due to the delivery of anti-cancer drug in the tumor, localized tumor heating under
109 the application of an external alternating magnetic field or laser, or controlled drug release in the tumor
110 following ultrasound exposure.

111 **GENERAL FABRICATION METHODS AND PROPERTIES OF IRON OXIDE** 112 **NANOPARTICLES**

113 To synthesize IONP, various chemical synthesis have been suggested, which involve: i) co-precipitation
114 by mixing ferrous and ferric salts in an aqueous medium, (Martínez-Mera2007), ii), electrochemistry
115 where an electric current is applied between an anode and a cathode introduced in an electrolyte, the
116 anode oxidizes metal ions of the electrolyte that are further reduced to metal by the cathode with the
117 help of stabilizers, (Khan2000, Ramimoghadam2014), iii), flow injection syntheses by mixing reagents
118 under laminar flow regime in a capillary reactor, (Salazar2006), iv), hydrothermal reactions in which
119 mixed metal hydroxides can be autoclaved to produce nanoparticle powders, (Wan2005), v), laser
120 pyrolysis where a laser heats a mixture of iron precursors and a flowing mixture of gas, (21
121 Verdaguer1998), vi), high temperature reaction of polyol with an iron source, (Cai2007), vii), sol-gel
122 methods in which precursors undergo hydroxylation and condensation to yield nanometric particles
123 (sol), followed by condensation and polymerization to produce a three-dimensional metal oxide network
124 (wet gel), ending by a heating process that results in a crystallized structure, (Albornoz2006), viii),
125 sonolysis or thermolysis involving the decomposition or collapse of organometallic precursors such as
126 ferrous salts, (Osuna1996), ix), spray pyrolysis in which solutions of ferric salts and a reducing agent in
127 organic solvent is sprayed in reactors leading to the condensation of the aerosol solute and solvent
128 evaporation, (Pecharroman1994).

129 Whereas IONP are predominantly composed of iron oxide, another material, which is often designated
130 as coating material, either surrounds the iron oxide core of IONP or is mixed with the iron oxide. Such
131 material is used to maintain IONP stability and enable its safe administration. A large number of
132 different coating materials have been added at the same time or following the fabrication of IONP core,
133 such as polysaccharides (Dias2011), acids (Laurent2008, Sahoo2005, boyer2010), polymers
134 (Boyer2010, Laurent2008, karimi2013), dendrimers (Walter2014, Parat2015), carbohydrate
135 (Mahmoudi2011), inorganic (Cui2014, Giakisikli2013) or organic materials (Gautier2013), metals
136 (Giakisikli2013), phosphates (Groult2014), silica (Mahmoudi2011, Alwi2012, Sun2005), dextran
137 (Osborne 2011, Hola2015, Berry2004), or PEG (Gupta2005, Hola2015). Furthermore, the coating
138 material usually contain a functional group, which is able to bind to the surface of IONP core, such as
139 OH (Hola2015, Boyer2010), NH₂ (Hola2015), COOH (Hola2015), thiol (Fauconnier1997), phosphonate
140 (Basly2010), or phosphate (Groult2014). Functions such as OH, NH₂, COOH, and thiol, usually give
141 rise to electrostatic interactions with the iron oxide, whereas phosphates yield covalent binding with the
142 iron oxide.

143 It goes beyond the scope of this article to describe in all details the various IONP physico-chemical
144 properties, such as IONP composition, size, charges, coating thickness, surface, interaction, geometry,
145 organization, distribution properties. The reader is redirected towards other excellent reviews on these
146 aspects, (Gupta2005, Laurent2008).

147 **COMMERCIALIZED OR CE MARKED FORMULATIONS CONTAINING IRON OXIDE**
148 **NANOPARTICLES FOR THE TREATMENT OF IRON ANEMIA DISEASE OR CANCER**
149 **TREATMENT:**

150 For patients suffering from iron anemia disease (IAD) for which orally administered iron does not lead
151 to sufficient efficacy, intravenous administration of the following IONP formulations have been
152 recommended and commercialized (Auerbach2017), (table 1):

- 153 • **Dexferrum** (also designated as iron dextran injection), consisting of high molecular weight iron
154 dextran complex at a concentration of 50 mg/mL mixed in solution with sodium chloride for tonicity,
155 (Dexferrum monograph),
- 156 • **Feraheme** (also designated as Ferumoxytol or Rienso), consisting of non-stoichiometric
157 magnetite superparamagnetic iron oxide nanoparticles (SPIO) of 16-31 nm and 750 kDa coated with
158 polyglucose sorbitol carboxymethylether, having a chemical formula $\text{Fe}_{5874}\text{O}_{8752}\text{-C}_{11719}\text{H}_{18682}\text{O}_{9933}\text{Na}_{414}$,
159 suspended in solution in the presence of mannitol at a pH of 6 to 8, an osmolality of 270-330 mOsm/kg,
160 and a concentration of 30 mg/mL, (Feraheme monograph),
- 161 • **Ferrisat** (also designated as Cosmofer, InFeD, iron dextran), made of a slightly viscous sterile
162 liquid complex of ferric hydroxide, dextran, and 0.9% sodium chloride, of pH 5.2-6.5, and concentration
163 of 50 mg/mL, (Infed monograph),
- 164 • **Ferrlecit** (sodium ferric gluconate complex in sucrose injection), made of a sodium salt of a
165 ferric iron gluconate complex in alkaline aqueous solution with approximately 20% sucrose w/v (195
166 mg/mL) and 0.9% w/v benzyl alcohol as preservative, of molecular formula
167 $[\text{NaFe}_2\text{O}_3(\text{C}_6\text{H}_{11}\text{O}_7)(\text{C}_{12}\text{H}_{22}\text{O}_{11})_5]$, MW 289-440 KDa, mixed in water at a pH of 7.7-9.7, concentration
168 of 12.5 mg/mL, administered at a minimum cumulative dose of 1 gram of elemental iron administered
169 over several sessions, (Ferrlecit monograph).
- 170 • **Monofer** (also designated as iron isomaltose), made of iron(III) atoms chelated with
171 carbohydrate, mixed in solution of pH of 5-7 with 0.9% sodium chloride, having a structure resembling
172 that of ferritin, designed to prevent the toxicity of unbound inorganic iron(III), and administered at a
173 dose, which is: i), lower than 1 g for fast administration, *i.e.* within more than 15 minutes or, ii) larger
174 than 1 g for a slow administration, *i.e.* within more than 30 minutes, (Monofer monograph),
- 175 • **Venofer®** (iron sucrose injection), made of a complex of polynuclear iron (III)-hydroxide in
176 30% w/v sucrose without any preservative, of MW ~ 34–60 kDa, proposed structural formula
177 $[\text{Na}_2\text{Fe}_5\text{O}_8(\text{OH})\cdot 3(\text{H}_2\text{O})]_n\cdot m(\text{C}_{12}\text{H}_{22}\text{O}_{11})$, where n is the degree of iron polymerization and m is the

178 number of sucrose molecules associated with the iron (III)-hydroxide, of iron concentration 20 mg/mL,
179 pH ~ 10.5, and osmolarity for injection ~ 1.250 mOsmol/L, (Venofer monograph),

180 • **Nanotherm®** is a CE marked IONP formulation, consisting of amino-silane coated SPIO of
181 diameter 15 nm, dispersed in water at an iron concentration of 112 mg/mL. It is designed to be
182 administered directly inside brain GBM tumor at a quantity of 200-600 mg of IONP and heated to 42-59
183 °C by applying an alternating magnetic field of frequency 100 kHz and strength 2.5–18 kA/m, (Maier-
184 Hoff2007, Maier-Hoff2011).

185 **PARAMETERS INFLUENCING THE BIODISTRIBUTION OF IRON OXIDE** 186 **NANOPARTICLES**

187 The different parameters that influence IONP biodistribution properties in the organism, which are
188 summarized in Figure 2, are the followings:

189 • **IONP size.** It first has an impact on IONP blood half-life ($t_{1/2}$). Indeed, it was shown that $t_{1/2}$
190 decreases from $t_{1/2} \sim 50$ min at 20 nm down to $t_{1/2} \sim 20$ min at 85 nm. (Kooi2003, Beaumont2009).
191 Second, it influences IONP route of elimination. IONP larger than 200 nm were reported to be degraded
192 by macrophages located in the marginal red pulp zone of the spleen that phagocyte IONP. IONP with
193 sizes lying between 200 nm and 10-15 nm can avoid renal clearance, diffuse through liver or spleen
194 fenestrated sinusoids and be trapped in these organs through macrophage phagocytosis (Feng2018).
195 IONP degradation in the liver is essentially carried out by Kupffer cells or hepatocytes, (Arami2015).
196 IONP captured in the liver are usually internalized by pinocytosis and degraded there, (Huang2010).
197 IONP smaller than 10-15 nm were reported to be captured and degraded by the kidney. The kidney
198 fenestrae act as filters that only allow IONP smaller than ~ 10–15 nm to leave the bloodstream and get
199 rapidly excreted from the body. Third, when IONP are used to treat an individual with a tumor, their
200 size determines their ability to enter (or not) the tumor through the enhanced permeability and retention
201 (EPR) effect. Small IONP can more efficiently than larger ones extravasate from the tumor blood
202 vessels by the EPR effect and diffuse in the tumor. The EPR effect is reported to occur for IONP with a

203 size lower than ~ 200-300 nm above which NP size becomes significantly larger than the size of the
204 blood vessel holes that irrigate the tumor, (Wang2017).

- 205 • **IONP hydrophobicity/hydrophilicity.** Hydrophobic IONP have a shorter circulation time (CT)
206 than hydrophilic ones since plasma proteins can more easily adsorb at their surface, yielding their
207 recognition by the reticuloendothelial system (RES), and removal from blood circulation, (Tong2010).
208 The core of IONP can be coated with hydrophilic molecules such as PEG to reduce opsonization and
209 increase IONP CT.
- 210 • **IONP surface charge.** It determines the efficacy of: i), adsorption of plasma proteins at IONP
211 surface leading to IONP recognition and capture by these cells, (Sakulku2014), ii), binding of IONP to
212 non-targeted cells yielding nonspecific IONP internalization, (Bachmann2002). Since both of these
213 mechanisms are enhanced for positively charged IONP, these IONP should yield a faster clearance
214 compared with negatively or neutrally charged IONP, although, to the author knowledge, this has not
215 been firmly demonstrated experimentally.
- 216 • **IONP core composition (maghemite versus magnetite).** Since degree of oxidation is relatively
217 similar between maghemite and magnetite, it is uncertain that it has a real impact on IONP
218 biodistribution profile.
- 219 • **IONP coating.** IONP are coated to enhance their stability, enable their administration, prevent
220 IONP capture by the immune system, or target specific organs. The strength of the interactions between
221 coating and core of IONP determines for how long IONP coating remains associated with the IONP
222 core *in vivo*, *i.e.* coating adsorption yields more rapid coating detachment than covalent binding of the
223 coating, (Arami2015). A general relation between IONP half-life values ($t_{1/2}$) and coating type can't
224 easily be deduced from experimental data due to the large variation of $t_{1/2}$ values reported for the same
225 coating material, *i.e.* 6 min to 21-30 h for dextran, 7 to 8 h for chitosan, 45 min to 62 h for PEG, 8 to 36
226 min for citrate, (Arami2015). The distribution in $t_{1/2}$ values may be attributed to different coating
227 thicknesses or types of interaction with IONP core observed for the same coating material. A specific
228 coating (inoleic acid, lactobionic acid, PEG, dextran, CMD) can prevent IONP opsonization and capture

229 by macrophages, and enable IONP to reach the liver or spleen, (Arami2015). Among the different types
230 of coatings, PEG has been the most widely used because of its stabilizing property via steric hindrance,
231 which prevents interaction with blood and serum proteins.

232 • **IONP administration route.** IONP injected: i), by inhalation, intrapulmonary, intratracheal, or
233 in intranasal route led to IONP retention in the lung without significant adverse effects (Lewinski2013),
234 ii) intravenously resulted in IONP capture by the RES as well as accumulation and/or excretion through
235 liver, spleen, and/or kidney depending on IONP size, (Arami2015), iii), intradermally yielded IONP
236 accumulation in regional lymph nodes, (Longmire2008), iv), orally led to IONP localization in the
237 gastrointestinal (GIT), where IONP with a specific coating can overcome the acidic environment of
238 GIT, diffuse through the liver without capture by Kupffer cells, and enter the general blood circulation
239 system, (Arami2015), v), intra-peritoneal resulted in IONP distribution in liver, lymph nodes and lung,
240 (Pham2018), vi), subcutaneously facilitated high tumor uptake, (Reddy2005), vii) intratumorally
241 resulted in IONP either rapidly leaving the tumor 3 hours following injection to migrate to the bone
242 (Zadnik2014) or remaining in the tumor more than 29 hours following injection (Kossatz2015).

243 • **Quantity of IONP administered in the organism.** When the quantity of IONP administered
244 (Q_{IONP}) is increased, the value of $t_{1/2}$ globally increases and IONP reach the liver at a later stage. Indeed,
245 as Q_{IONP} increased from 0.0145-0.224 mg/kg to 11-15 mg/kg, $t_{1/2}$ was reported to globally increase from
246 1-81 to 13-37200 minutes. Furthermore, while for $Q_{\text{IONP}} \sim 15 \mu\text{mol Fe.kg}^{-1}$, IONP reached the liver 1-4
247 h following IONP injection, they accumulated in this organ at a later stage for $Q_{\text{IONP}} \sim 150 \mu\text{mol Fe.kg}^{-1}$,
248 *i.e.* 8-24 h following IONP administration (Arami 2015).

249 • **IONP geometry.** Indeed, it was reported that nanoparticles with a large length to width aspect
250 ratio, (Geng2007), possess a longer blood circulation time than their spherical counterparts,
251 (Petros2010). IONP geometry could also possibly determine the organ in which nanoparticles diffuse,
252 with elongated and spherical nanoparticles accumulating predominantly in lymph nodes, (Park2008),
253 and liver, (Zhao2013), respectively.

254 • **IONP opsonization.** It is another important factor determining the toxicity/biodistribution of
255 these nanoparticles. Opsonization mechanism, which was reported to occur both at IONP core and
256 coating surfaces, seems to depend on: i), protein molecular weight and IONP size with heavier proteins
257 seemingly adsorbing onto larger IONP, (Sakulku2014), ii), charges with proteins adsorbing onto IONP
258 surface of opposite charge as that of proteins. For IONP coated with dextran, cationic plasma proteins
259 such as histidine-proline rich glycoprotein (HPRG) and high molecular weight kininogen (HMWK)
260 were observed to bind to anionic magnetite cores, (Simberg2009), while immunoglobulins (IgG) and
261 mannanbinding lectins (MBL) were observed to interact with the cationic dextran coating,
262 (Simberg2009). In general, opsonized IONP were shown to yield longer $t_{1/2}$, CT, and/or clearance
263 values, (Arami2015).

264 **IONP BIODISTRIBUTION AND PHARMACOKINETICS**

265 IONP biodistribution properties depend on the physiological barriers that they encounter, their faculty to
266 cross (or not) these barriers as well as the chosen administration route (see previous section).
267 Biodistribution properties are summarized in table 2 for IONP administered by intravenous, intra-
268 gastric, or intraperitoneal route, to mice, rats, and pig, at a dose comprised between 0.5 to 2000 mg/Kg.
269 When IONP are injected intravenously, they can be captured by white blood cells such as monocytes
270 and residential tissue macrophages, and accumulate in liver and spleen, (Feng2018). Redistribution in
271 these organs depends on the following parameters: i), the time following administration, *i.e.* it was
272 observed to increase during 5-15 hours following IONP injection and then decrease afterwards
273 (Azadkbakht2017), ii), the size of IONP, the smallest (10 nm) and largest (40 nm) IONP were reported
274 to accumulate predominantly in the liver and spleen, respectively (Yang2015), iii), the quantity of
275 IONP administered with IONP possibly distributing in spleen after saturation of the liver, (Remya2016).
276 In the liver, IONP are phagocytized by Kupffer cells, which degrade and metabolize them partly or fully
277 in dissolved iron and/or in a protein-iron complex, called ferritin, possibly with the help of liver
278 hepatocytes (Gu2012, Briley-Saebo2004). When Kupffer cells are saturated by a too large quantity of
279 IONP, (Arami2015), IONP could be degraded by spleen macrophages. IONP can be found in smaller

280 quantity than in spleen and liver in other organs such as lung, kidney, heart, bladder, muscle, ovary,
281 colon, muscle, pancreas, intestine, stomach, and uterus, (table 2). When IONP enter an organ, they
282 ultimately may diffuse to the lymph nodes surrounding it, (Thorek2006). Under specific conditions in
283 terms of IONP size, coating, and presence of a specific targeting compound, IONP have been reported
284 to cross several physiological barriers such as the blood brain, (Huang2016), placental (Muller2018), or
285 skin barrier (Musazzi2017). Furthermore, IONP can also target tumors following intravenous
286 administration, either through passive targeting also called enhanced permeability retention (EPR)
287 effect, (Maeda2010), or active targeting using a compound attached to IONP such as peptides (*e.g.*
288 chlorotoxin, RGD, CREKA, bombesin, F₃, A₅₄, LHRH), antibodies (*e.g.* Anti-HER₂, Anti-
289 EGFR/EGFRvIII), and small molecules (*e.g.* folate) that can specifically recognize tumor cells,
290 (Cole2011). Concerning IONP excretion mechanism, although it was suggest that the largest IONP end
291 up in liver, spleen and then feces while the smallest ones are eliminated through kidney and urines,
292 multiple IONP transformations in the organism can possibly yield a different behavior.

293 **TARGETING MECHANISMS OF IRON OXIDE NANOPARTICLE**

294 **Passive targeting (EPR effect):**

295 The efficacy of passive targeting, measured by estimating the percentage of injected IONP in tumors
296 resulting from passive targeting of various types of IONP (different charges, coatings, encapsulations,
297 compositions), following intravenous injection of 0.1-4 mg of IONP to mice suffering from different
298 types of tumors, is summarized in table 3. The EPR effect is a consequence of angiogenesis, which leads
299 to highly proliferating endothelial cells with a low density, and to openings of 100-800 nm between
300 these cells. Nanoparticles, which are smaller than 100-800 nm can extravasate or diffuse from the blood
301 vessels into the tumor interstitium. On the one hand, the largest nanoparticle size for which the EPR
302 effect occurs seems to be ~ 200 nm, since nanoparticles larger than 200 nm could be captured by the
303 spleen or liver and not able to reach the tumor. On the other hand, nanoparticles smaller than 30 nm
304 could diffuse back from the tumor to the blood vessel, and be eliminated by the MPS or kidneys,
305 (Sun2014). The range of nanoparticle sizes that yields the most efficient tumor retention is therefore

306 comprised between 30 and 200 nm. Other nanoparticle parameters can have an impact on EPR efficacy
307 such as : i), the shape of the nanoparticles with spherical nanoparticles apparently diffusing less
308 efficiently through the vascular wall than rod- and bar-shaped nanoparticles, ii) the sleath capacity of the
309 nanoparticles, provided for example by the presence of PEG molecules at nanoparticle surface, leading
310 to prolonged circulation half-life, less protein adsorption, reduction in clearance by the MPS, and thus
311 improved tumor accumulation, iii) the charge of nanoparticle with slightly negatively charged
312 nanoparticles escaping from macrophage endocytosis, and therefore more efficient accumulating in
313 tumor, (Sun2014). To be more efficient, passive targeting needs to overcome the following limitations:
314 i), the in-homogeneous distribution of blood vessels resulting from angiogenesis that yields non-uniform
315 permeability within the whole tumor, ii), its limited efficacy on small tumors or metastases that display
316 reduced angiogenesis, iii), its efficacy of tumor targeting leading to 0.0005-3 % of injected IONP in
317 tumor (table 3) and to 20-30% more nanoparticles in tumors compared with other organs,
318 (Kobayashi2014). While the EPR effect was reported to lack efficacy in some studies, (Wilhelm2016),
319 it was described as enabling nanoparticles to achieve much improved targeting efficacy compared with
320 other drugs in some other studies, (Golombek2018).

321 **Molecular targeting:**

322 Active targeting usually occurs after nanoparticles have diffused to the tumor by passive targeting,
323 making the efficacy of active targeting dependent on that of the EPR effect. The principle of active
324 targeting relies on interactions between a ligand attached to the nanoparticles and a receptor located at
325 cell surface. Examples of ligands are: i) various monoclonal antibodies, *e.g.* 610, L6, HER/Neu, A7, and
326 antibody to prostate specific membrane antigen, ii) transferrin, iii) various peptides , *e.g.* EPPT,
327 Chlorotoxin, F3, and CREKA, iv) folic acid and methotrexate, v) Herceptin, vi) RGD, vii) luteinizing
328 hormone releasing hormone (LHRM), which target: i) antigens of different tumor cells, ii) transferrin
329 receptor, iii) Underglycosylated mucin-1 antigen (uMUC-1), membrane-bound
330 matrixmetalloproteinase-2 (MMP-2), Surface-localized tumor vasculature, Clotted plasma proteins, iv)
331 folate receptor, v) Her-2/neu receptors, vi) $\alpha_v\beta_3$ integrins, and vii) LHRH receptor, respectively,

332 (Peng2008). To be efficient, active targeting seems to require the combination of a rather larger number
333 of properties such as: a larger quantity of receptors on cancer than non-cancer cells, the availability of
334 receptors on cancer cell surface, the successful binding of ligands to receptors followed by
335 internalization of the complex ligand-IONP in cancer cells, an homogenous distribution of receptors
336 within the tumor, the availability of cancer cells used for *in vitro* assessment of ligand/receptor
337 interactions displaying similar properties than cancer cells found in a patient's tumor, a sufficiently
338 large fraction of tumor cells expressing a receptor specific to the used ligand. Table 4 summarizes the
339 efficacy of active targeting reached by administering intravenously to mice suspensions containing
340 between 0.073 and 0.5 mg of IONP combined with different types of targeting agents (TA), *i.e.* various
341 antibodies (PSMA antibody, anti-GD2 antibody, Trastuzufab, antibody fragment Ffab), biotin, folate,
342 and RGD. It was reported that the administration of IONP with TA increases the percentage of IONP in
343 tumor, *e.g.* the percentage of injected IONP increases from 1.4% without Trastuzufab to 3% with
344 Trastuzufab, (Dong2015). Interestingly, such improvement was observed for IONP of the lowest size
345 (30 nm), and not for those of 100 nm, suggesting that as for the EPR effect, the efficacy of active
346 targeting may depend on nanoparticle size, (Dong2015). Another interesting study has shown that by
347 adding carboxy-methyl-dextran (CMD) at the surface of the nanoparticles, which increases IONP
348 circulation time, the efficacy of active targeting of IONP toward KB tumors increases from 4% using
349 IONP associated with Ffab to 7% using IONP combined with Ffab and CMD. Due to the large number
350 of parameters that needs to be under control to make active targeting efficient, it is unsurprising that
351 various studies report very different efficacy for active targeting (table 4). As a whole, it however
352 appears that active targeting is promising and therefore deserves to be tested on humans.

353 **Magnetic targeting:**

354 The principle of magnetic targeting relies on the application of a magnetic field gradient that results in a
355 magnetic force, F , which is sufficiently strong to drive IONP towards the tumor, usually in an opposite
356 direction from that of the blood flow. Using a simplified approach that does not take into consideration
357 the complexity of biological systems but gives an idea of the parameters onto which the magnetic force

358 depends, it was suggested that: $F = M_s \cdot V \cdot \nabla B$, where M_s and V are the saturating magnetization and
359 volume of IONP, respectively, and ∇B is the magnetic field gradient applied on IONP,
360 (Bietenbeck2016). According to this relation, the magnetic force is the strongest when the saturating
361 magnetization, volume of nanoparticles, and ∇B reach the largest values. In fact, although the values of
362 these three parameters should be large enough for magnetic targeting to be efficient, they can't exceed
363 certain values, *e.g.* first the volume of IONP should remain below the volume at which IONP switch
364 from a single to a multi domain magnetic behavior, second the saturating magnetization may be
365 increased by doping iron oxide nanoparticles with various materials such as cobalt but these materials
366 are usually toxic and can't easily be used for medical application, third too large magnetic gradients
367 should be avoided since they could also possibly prevent efficient targeting. The efficacy of magnetic
368 targeting further depends on other parameters such as blood flow or viscosity, and SPION concentration
369 in the blood. Regarding the properties of the magnetic field required to reach efficient magnetic
370 targeting, most studies report the use an external and static magnetic field to guide IONP toward the
371 tumor region, whose strength is between 0.2 and 0.6 T. The value of the magnetic field gradient, which
372 is more directly linked to the efficacy of magnetic targeting than that of the magnetic field strength, is
373 usually not given. One study mentions the use of a magnetic field gradient of 100 T/m to drive IONP
374 through arteries, (Bietenbeck2016). Interestingly, efficient magnetic targeting was observed for different
375 durations of application of the magnetic field, which was typically comprised between 30 min,
376 (Aguiar2017) and 48 h (Estelrich2015). Practically, for efficient magnetic targeting, different types of
377 magnets such as neodymium magnet or electromagnet, (Aguiar2017), can be attached at the surface of
378 the skin, located above the tumor of the treated animals. Table 5 summarizes the efficacy of magnetic
379 targeting of various types of IONP (different coatings, charges, compositions) injected intravenously or
380 intra-arterially to mice or rats at a dose comprised between 0.3 mg and 4 mg per animal, under the
381 application of a magnetic field usually orientated in the direction of the tumor of strength comprised
382 between 0.32 T and 1.2 T. It was shown in several studies that magnetic targeting improves the efficacy
383 of targeting, *i.e.* the percentage of injected IONP ending up in the tumor increases from $5 \cdot 10^{-3}$ to $5 \cdot 10^{-2}$

384 2% for G100 starch coated IONP targeting 9L glioma tumor, from 2 to 6% for IONP encapsulated in
385 nanocapsule targeting CT-26 colon tumor, from 0.1 to 0.5-0.8% for IONP coated with amino groups
386 targeting C6 glioma tumors, from 3 to 7% for IONP embedded in nano-bubbles targeting CT26 colon
387 tumors, and from 5 to 12% for IONP of composition ZnMnFeO targeting 4T1 tumor (table 5).
388 Interestingly, it was also reported that several IONP properties can further improve the efficacy of
389 magnetic targeting such as: i) the application of focused ultrasound (FUS) for the targeting of C6 glioma
390 brain tumors that possibly favors the diffusion of IONP to brain tumor by opening the blood brain
391 barrier, resulting in an increase in the percentage of injected IONP in the tumor from 2% using MT
392 without FUS to 6% using MT with FUS, ii) the coating surrounding IONP that can increase the
393 circulation time of IONP or contribute to an active targeting mechanism, resulting in an increase of the
394 percentage of injected IONP in the tumor from 1% using IONP without a specific coating to 3% using
395 IONP coated with β -glucosidase that targets amygdalin and furthermore to 6.5% using IONP coated
396 with both β -glucosidase and PEG (Zhou2014). As a whole, the percentage of injected IONP that ends
397 up in the tumor following magnetic targeting varies quite a lot depending on the tested condition and
398 lies between 5.10^{-3} and 2.6%. Given the values of these percentages, the majority of IONP does not end
399 up in the tumor but in other parts of the organism, and one should therefore verify that despite the small
400 values of these percentages, IONP are located in a sufficiently large quantity in the tumor to be able to
401 trigger anti-tumor activity.

402 **MECHANISME OF ACTION OF IONP IN TREATMENT OF IRON ANEMIA DISEASE**

403 IONP can be used for the treatment of iron anemia disease, possibly associated with chronic kidney
404 disease (CKD), and combined (or not) with other drugs such as erythropoietin. They are usually
405 prescribed when oral administration of iron based drugs is not sufficiently efficient. IONP can be
406 administered using a typical minimum total dose of 1 g of elemental iron. Sequential sessions can be
407 used to increase the quantity of injected IONP. The mechanism of action of these IONP relies on the
408 capture of these nanoparticles by the RES, which is believed to separate iron from other materials
409 comprised in IONP (dextran for dexferrum and ferrisat, polyglucose sorbitol carboxymethylether for

410 feraheme, gluconate for ferrlecit, carbohydrate for monofer, amono-silane for nanotherm) and then
411 make iron bio-available for the organism. These IONP were reported to mainly distribute in blood,
412 extravascular fluid, liver, spleen and bone marrow. Iron could be trapped and possibly slowly released
413 in the bone marrow, liver, and/or spleen, and then get bound to or form hemosiderin, ferritin, or
414 transferrin, which can store or transport iron in the organism. This treatment was also described as
415 increasing the hemoglobin concentration in the organism. These IONP are characterized by large $t_{1/2}$
416 values, *i.e.* $t_{1/2} \sim 59$ hours for dexferrum, $t_{1/2} \sim 15$ hours for Feraheme, $t_{1/2} \sim 5-20$ hours for Ferrisat, $t_{1/2} \sim$
417 7-12 hours for Injectafter, $t_{1/2} \sim 1-4$ days for Monofer, $t_{1/2} \sim 6$ h for Venofer, (monographs of these
418 various drugs). Iron originating from these IONP usually displays negligible elimination by the kidneys.
419 Other material than iron, such as IONP coating, can be excreted through urines and/or feces or be
420 metabolized. The low toxicity of these IONP was highlighted by large LD_{50} values in mice, *e.g.* above
421 500 mg/kg (Monogr. Dexferrum).

422 **IONP FOR DELIVERY OF ANTI-CANCER DRUGS IN THE ABSENCE OF AN EXTERNAL** 423 **SOURCE OF EXCITATION**

424 First, IONP may be used to increase the probability of success of cancer gene therapy. In this case,
425 IONP can be coated with polymers, such as PEI, PEG, or chitosan, or made positively charged to bind
426 IONP with negatively charged nucleic acids, belonging either to DNA plasmids or to siRNA. The use of
427 IONP favors cellular internalization of DNA or siRNA, which further promotes transfection of DNA or
428 diffusion of siRNA in the cytoplasm followed by the inhibition of mRNA translation. In this way, the
429 normal behavior of cells could be restored by delivering DNA plasmids that can replace damaged genes
430 or siRNA that can prevent the expression of oncogenes, (Kievit2011).

431 Second, another application of IONP is the treatment of cancer by protein therapy. IONP can be
432 associated or linked to proteins to favor the antitumor mechanisms such as: i) the blocking of cell
433 surface receptors by using for example III (EGFRvIII) antibody that inhibit the cellular receptor
434 EGFRvIII, (Hadjipanayis2010), ii) CTX that decreases tumor cell proliferation, (Velseh2009), iii)
435 Cytochrome c that favors tumor cell apoptosis, (Santra2010), iv) interferon gamma ($IFN\gamma$) that triggers

436 an anti-tumor immune activity, (Mejias2008). Compared with the use of free proteins (without IONP),
437 proteins associated with IONP should be less easily metabolized or cleared, be delivered more
438 efficiently to cancer cells, be protected from protease degradation, or more efficiently interact with parts
439 of cancer cells such as cell receptors by being located on IONP surface.

440 Third, IONP can enhance chemotherapy efficacy. As for gene and protein therapy, the underlying
441 mechanism relies on enhanced interaction between chemotherapeutic drugs and cells by linking these
442 drugs to IONP. By using IONP, chemotherapeutic drugs can have a better access to several parts of the
443 cells such as the cell nucleus to inhibit DNA replication, or mitochondria to prevent mitochondrial
444 activity. As an example, IONP conjugated to the anti-cancer drug MTO was administered intra-arterial
445 into rabbits, leading to drug accumulations in the tumor, complete tumor remissions, and to an apparent
446 cure among 30% of treated animals, (Tietze2013).

447 IONP and chemotherapeutic drugs can be linked together in different ways, (El-Boubbou2018), *i.e.*
448 through:

- 449 • Electrostatic, dipole–dipole, or van der Waals forces interactions between drugs and IONP,
450 resulting in IONP-drug complexes that are relatively simple to fabricate, prevent drug covalent
451 modification, and enable a control of drug delivery in the desired target of the organism,
- 452 • Drug encapsulation, *e.g.* by coating IONP core with a porous material such as silica, in which
453 drugs can be inserted, or by making phospholipid bilayers inside which IONP can be inserted,
454 preventing drug degradation, limiting drug side effects, and yielding controlled drug release.
- 455 • Covalent binding between IONP and drugs, *e.g.* using amine functionalized IONP that are
456 covalently conjugated to MTX, and enable drug release at an acidic pH of 2, (Kohler2005).

457 Compared with non-covalent bindings, covalent ones present both advantages and drawbacks. On the
458 one hand, they are very strong and can usually more efficiently resist physiological disturbance and
459 therefore yield a larger life time in the bloodstream. On the other hand, they can be non-biocompatible
460 depending on the chemicals used for the binding, non-cleavable after IONP have reached their target, or
461 enable only a small quantity of drugs attached to each IONP.

462 **IONP TRIGGERING ANTI-CANCER ACTIVITY IN THE PRESENCE OF AN EXTERNAL** 463 **SOURCE OF EXCITATION**

464 The most widely used energy source to excite IONP has been the alternating magnetic field, in a
465 treatment method called magnetic hyperthermia, (Obaidat2015, Chang2018, Giustini2010, Perigo2015).
466 When IONP are exposed to such magnetic field, it either produces the rapid inversion of the magnetic
467 moment or physical rotation of IONP. Both moderate heating, at typically 41-46 °C, and other non-
468 thermal effects, such as nanoparticles movements, have been reported to result from such excitation and
469 yield anti-tumor activity. Typical values of the magnetic field strength and frequency that need to be
470 applied to produce efficient magnetic hyperthermia are larger than 5-20 mT and 50-100 kHz,
471 respectively. In general, the strength and frequency of the magnetic field should remain below 20 mT
472 and 100 kHz to avoid non-localized heating that can occur outside of the nanoparticle regions, due to
473 Foucault currents produced by AMF of large strength and frequency. IONP have been administered
474 intra-tumorally or intravenously to treat different types of mouse tumors, and produced anti-tumor
475 activity, (Hayashi2013, Huang2013). Clinical trials are ongoing on patients suffering from glioblastoma
476 to evaluate the efficacy of this treatment, (Maier-Hoff2007, Maier-Hoff2010).

477 The laser is another excitation source that can be applied on IONP to heat the tumor. A laser wavelength
478 of 808 nm, which yields minimal laser absorption by tissues, as well as a laser power density of
479 typically 1-5 W/cm², were reported to yield efficient heat production by IONP, (Esterlich2018). As an
480 example of the efficacy of such treatment, when C6 tumors were subcutaneously grown under the skin
481 of mice and injected with 0.2 mg of IONP followed by 5 minutes tumor laser exposure (825 nm, 1.5
482 W/cm²), it prevented tumor growth, (Wang2018).

483 In the case of ultrasound tumor treatment, it was also suggested to use IONP combined with a
484 sonosensitizer to trigger anti-tumor activity, (Qian2016). Indeed, when SkBr3 breast tumors of 100 mm³
485 were injected intravenously with 0.4 mg of IONP conjugated with PEG and Rose Bengal sonosensitizer
486 followed first by the application of a magnetic field on IONP to enhance tumor targeting and then by
487 tumor ultrasound irradiation at 2 W during 60 seconds 24 h post injection, it led to an increase of the

488 tumor temperature from 31 °C to 48 °C and to a decrease of tumor size within one month following
489 treatment (Chen2016).

490 **CONCLUSION**

491 In this review, the different methods of IONP fabrication as well as the various IONP physico-chemical
492 properties are briefly presented. IONP are currently commercialized or CE marked for treatments of
493 iron anemia or cancer diseases. For these applications, they can be administered to patients, at doses that
494 can exceed 1 gram per patient in some specific conditions.

495 The different parameters that influence iron oxide nanoparticle toxicity and biodistribution are:

- 496 • **IONP size.** It that has an impact on: i) $t_{1/2}$ values, *i.e.* $t_{1/2}$ increases with decreasing IONP sizes,
497 ii), the route of elimination, *i.e.* IONP > 200 nm are degraded in spleen, 10 nm < IONP < 200 nm are
498 eliminated in liver and spleen, while IONP < 10 nm are excreted through kidney.
- 499 • **IONP Hydrophobic/Hydrophilic properties.** Hydrophobic IONP yields a shorter circulation
500 time than hydrophilic ones.
- 501 • **IONP surface charge.** Positively charged IONP have a faster clearance than negatively or
502 neutrally charged ones.
- 503 • **IONP coating.** IONP specific coating, such as PEG, can prevent nanoparticle capture by
504 macrophages.
- 505 • **IONP administration route.** It determines in which organ IONP distribute and how they are
506 eliminated.
- 507 • **Opsonization.** When IONP are opsonized or when their quantity administered increases, it can
508 lead to an increase in $t_{1/2}$ values.

509 Concerning the use of IONP for the treatment of iron anemia, it necessitates a sufficiently large IONP
510 circulation time to enables efficient IONP capture by the RES followed by IONP dissolution into free
511 iron. This can be achieved by choosing appropriate IONP properties, in particular IONP coating and
512 IONP size. With regard to cancer treatment with IONP, it necessitates that a sufficiently large quantity

513 of IONP reaches the tumor. For that, different types of targeting strategies have been tested. Although
514 molecular and magnetic targeting do not seem to improve in all cases targeting efficacy compared with
515 passive targeting, it seems that the quantity of IONP in the tumor is sufficient to trigger anti-tumor
516 activity using IONP for the delivery of chemotherapeutic drugs in the tumor, localized heat in the tumor
517 following the application of an alternating magnetic field or laser, or the activation of a sonosensitizer
518 under ultrasound exposure.

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972 **FIGURES:**

973 **Figure 1:** A schematic picture representing two different types of applications of iron oxide
974 nanoparticles for treatments of iron anemia disease and cancer.

975 **Figure 2:** The different properties of iron oxide nanoparticle properties influencing their biodistribution.

976 **TABLE:**

977 **Table 1:** Commercialized or CE marked iron oxide nanoparticles for treatments of iron anemia disease
978 and glioblastoma.

979 **Table 2:** Biodistribution properties and pharmacodynamics parameters of various iron oxide
980 nanoparticles.

981 **Table 3:** Percentage of injected IONP ending up in tumor through the EPR effects when different
982 quantities of IONP are administered intravenously to mice or rats bearing different types of tumors.

983 **Table 4:** Percentage of injected IONP ending up in tumor through molecular targeting when different
984 quantities of IONP conjugated to various targeting agents are administered intravenously to mice
985 bearing different types of tumors.

986 **Table 5:** Percentage of injected IONP ending up in tumor through magnetic targeting targeting when
987 different quantities of IONP are administered intravenously to mice bearing different types of tumors
988 and the tumor is exposed to a magnetic field of various strengths.

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Two medical applications of iron oxide nanoparticles

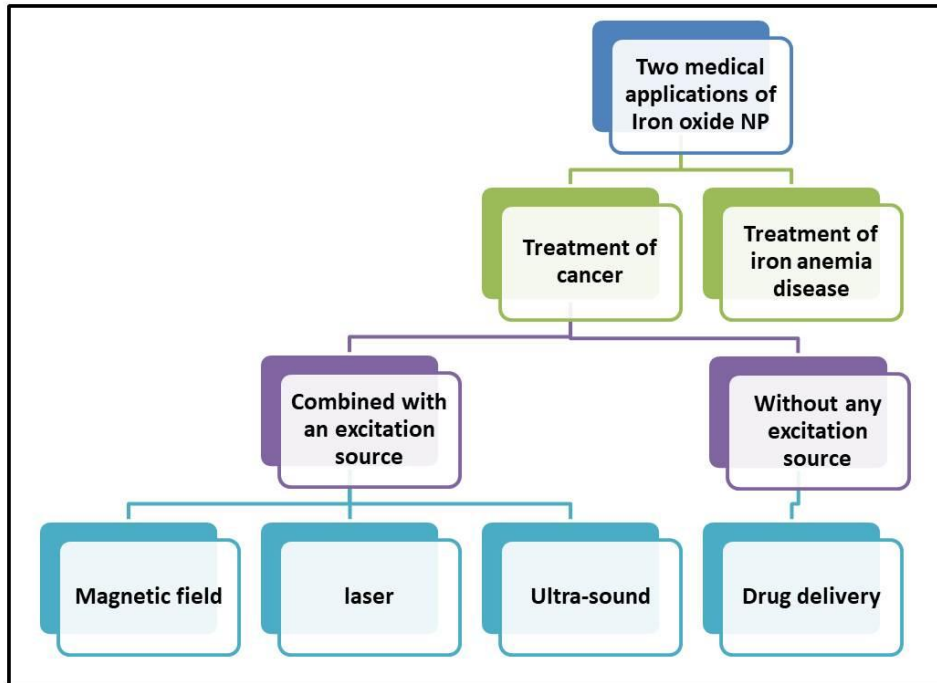


Figure 1

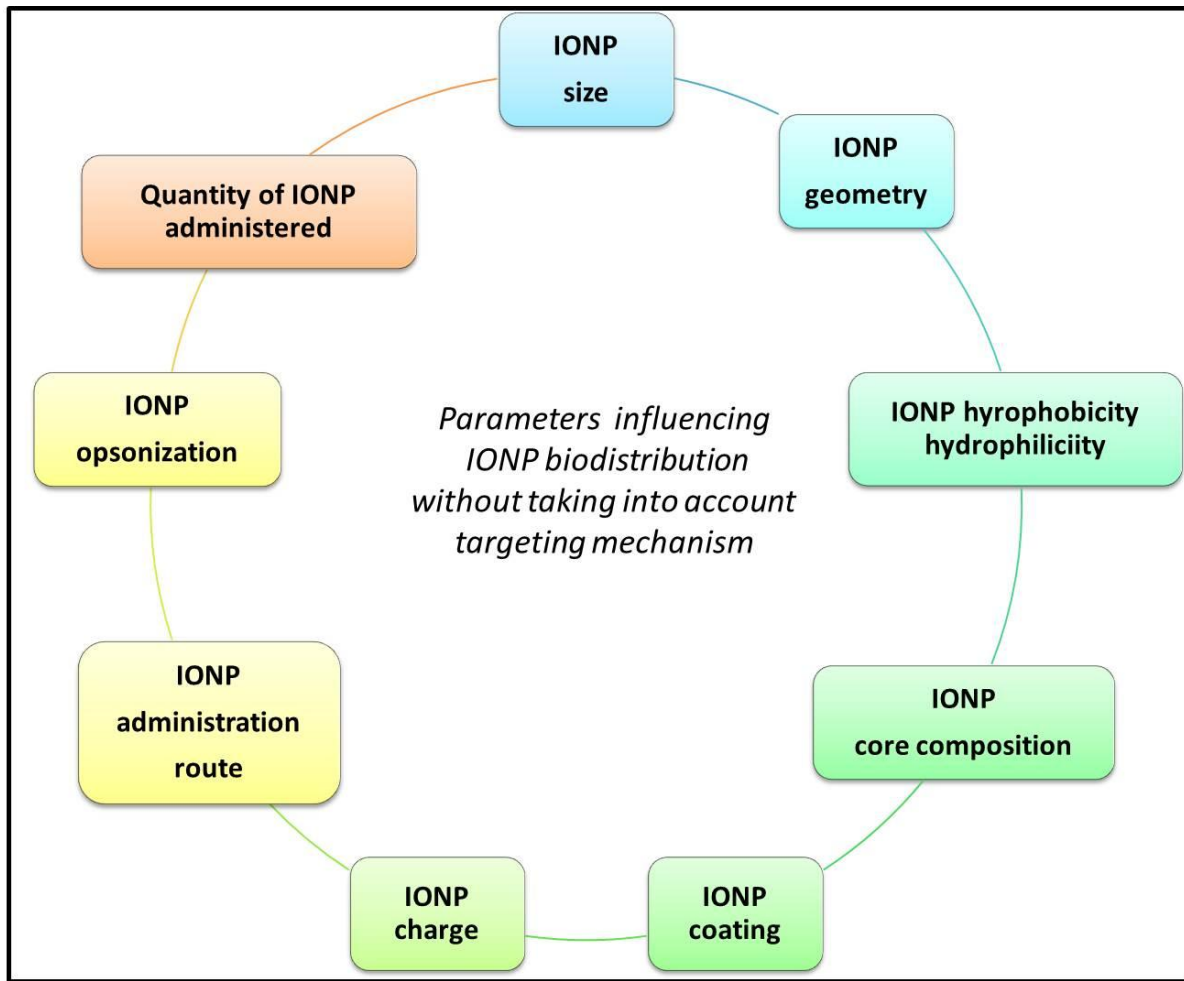


Figure 2

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| Product name | Product type | Status | Application |
|-----------------------------------|--|----------------|---------------------------------------|
| Feraheme, Ferumoxytol, Rienso | FeOCHONa NP + polyglucose sorbitol carboxymethyl ether coating | Commercialized | Treatment of IDA in patients with CKD |
| Ferrisat | Iron + Dextran | Commercialized | Treatment of IDA |
| Venofer | Iron + sucrose | Commercialized | Treatment of IDA in patients with CKD |
| DexFerrum, iron dextran injection | Iron + dextran | Commercialized | Treatment of IDA in patients with CKD |
| Injectafter Ferinject | Sodium ferric gluconate | Commercialized | Treatment of IDA in patients with CKD |
| Nanotherm | amino-silane coated iron oxide nanoparticle | Commercialized | Magnetic hyperthermia treatment |

Table 1

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Biodistribution/pharmacodynamic parameters of Fe₃O₄/γFe₂O₃ NP

| NP size (nm) name | Coating type | Quant. Admin. | Admin. Route | Animal | Organs with large NP accumulation | Organs with low NP accumulation | Elimination route | Pharma. paramet. | Remarks | Ref (first aut. name/year pub.) |
|--|-------------------------------------|----------------|---------------|----------------------|--|---|--|--|---|---------------------------------|
| 5, 15, 30 (TEM) 13, 30, 50 (hydro) 12-15 | PEG | 5 mg/kg | iv | Mice | Liver, spleen (24h) | Lung, kidney, brain (24h) | Liver and spleen Degraded products in blood | t _{1/2} = 200 min (5 nm) t _{1/2} = 63 min (30 nm) | Decrease in half life with increasing sizes | Gu2012 |
| 11 | DMSA | 0.5-2 mg/kg | iv | pig | Liver, spleen | Lungs, | Liver (no accumulation in kidney) | t _{1/2} = 15 min. | Decrease in blood pressure after NP admin. | Edge2016 |
| 17-46 (hydro) | OA + pluronic | 10 mg/kg | iv | Rats | Liver, spleen | Brain, heart, kidney, lung | 18-22% of NP through urine and feces during 7 weeks | Clearance > 3 weeks | None | Jain 2008 |
| 32-46 (hydro) | PEG Dextran | 5 mg/kg | iv | mice | Liver, kidney (0-50 h. after amin.) | Bladder, muscle (0-50 h) | Through Liver Renal clearance | NA | Very different biodistribution profile compared with Gd | Leal 2015 |
| 10-25 | PEG | 100 mg/kg | iv | mice | Liver, spleen (24 h. after inj) | NA | Spleen and/or liver | t _{1/2} < 1h | NP in blood during 24 h. | Mohamaddi 2018 |
| 25 | PEG | 90 mg/kg | ip | Mice (ovarian tumor) | Liver, spleen, omentum, mesentery (0-1 week) | Bladder, brain, colon, heart, left kidney, left ovary, lungs, peritoneum, stomach, uterus | Excretion in feces (1h-1 week) Spleen and liver macrophages | NA | More NP in tumor for larger SPION (1-24 h after admin.) | Pham 2018 |
| 12 | Dextran | 300-2000 mg/kg | iv | Mice | Liver, Spleen | kidney | lack of defined pathway for excretion | NA | Iron accumulation in liver, spleen, kidney does not induce tox. | Remya 2016 |
| 10 (TEM) | PEG | 2.5-5 mg/kg | iv | Rats | Liver (3 h) | Spleen, lung (3 h) | Liver assimilation of iron Excretion from liver in 200 h. | t _{1/2} = 20 min | NP excreted in the form of free iron. | Ruiz 2013 |
| 41 (hydro) | dendrimer | 250 mg/kg | ip | Mice (tumor) | Liver, lung, heart, kidney, tumor, heart (4-24 h) | NA | Excretion through kidney and absorption in lungs | t _{1/2} < 24h | Unusual mode of excretion | Salimi2018 |
| 29 (hydro) | dextran | 50 mg/kg | iv | Mice | Liver (< 24h) Heart (< 5 min) Lung (<60 min) | Kidney, adrenal, pancreas, tail, carcass | Feces excretion negligible. NP accumulate in liver + spleen | t _{1/2} < 5 min | Gradual increase of NP accumulation in spleen (peak at 60 min) | Shanehsazadeh 2013 |
| 20 (TEM) | EDT Ethylenediaminetriacetate | 600 mg/kg | iv | Mice | Liver (< 30 min) | Spleen, kidney (<30 min) | Essentially liver (possibly a little bit through kidney) | t _{1/2} = 6 min | Can cross the BBB due to the coating LPA | Sun2016 |
| 2.5-7 (TEM) | None | 12 mg/kg | Intra-gastric | Mice | Liver, Spleen (> 10 days) | Heart, lungs, kidneys, brain, stomach, small intestine (> 10 days) | Renal excretion modest | t _{1/2} > 10 days | IONP can cross the BBB | Wang2011 |
| 20 (TEM) | ZDS Zwitterionic dopmanie sulfonate | 600 mg/Kg | iv | Mice | Liver, carcass | GIT, Kidney, Spleen, lung, heart, tail (24 h) | After 24 h, majority of iron from NP in urines | NA | Renal clearance due to small size of IONP | Wei2017 |
| 10, 20, 30, 40 | NA | 600 mg/Kg | Intra-gastric | Mice | Liver, spleen | Heart, lungs, kidney, brain, stomach, small intestine | Modest renal excretion | NA | Presence of IONP in all organs | Wang2010 |
| | carboxyl | 20 mg/Kg | iv | Mice | 10 nm IONP: liver 40 nm IONP: Spleen | stomach, intestine, and uterus | All IONP in Feces Small IONP (10 nm) in urine | NA | Biodistribution and elimination depends on size of IONP | Yang2015 |

Table 2-1

| IONP size (nm) name | Not Fe203 Coating / drug | Quant. Admin. | Admin. Route | Animal | Organ with larger NP accumulation | Organs with lower NP accumulation | Elimination route | Pharma. paramet. | Remarks | Ref (first aut. name/year pub.) |
|-----------------------|--|-----------------|--------------|--------------------------|---|--|---|---|---|---------------------------------|
| 20 (TEM) | PEG | 10 mg/kg | iv | Mice | Liver, spleen (24h, 72h) | Kidney (24h, 72h) | NA | NA | NA | Arami 2015 |
| NA | Chitosan + Chlorotoxin (targeting) | 10 mg/kg | iv | Mice | Liver, spleen, kidney (0-48h) No diff. with/without targeting | Colon, brain, heart, lung, pancreas, muscle, small intestine, gonad, bone marrow (0-48h) No diff with/without targeting | Kidney and liver | t _{1/2} = 8 h (with targeting) t _{1/2} = 7 h (without targeting) | Longer circulation with targeting molecule that prevents elimination. | Lee 2010 |
| 5 (TEM) 10-20 (hydro) | PEG RGD-modified | 5 mg/Kg | iv | Mice bearing HepG2 tumor | Liver, spleen (not a big difference between blank, PEG, RGD-modified) | Heart, lung, kidney, tumor | liver | t _{1/2} = 92 min (RGD) | For heart, liver, spleen, lung, kidney): No difference in biodistribution between blank, PEG-IONP or RGD-IONP For tumor: No difference between IONP-RGD and IONP-PEG | Jia2016 |
| 120 (hydro) | DOXO + RGD (targeted) DOXO (non-targeted) | 0.5 mg/kg | iv | Mice bearing CNS-1 GBM | Non-targeted organ: distribution in liver, spleen, heart, brain, kidney | | Organs of the RES | NA | Non-targeted IONP: 2% in tumor Targeted IONP: 4.5% in tumor | Karathanasis2016 |
| 8-10 (TEM) 66 (hydro) | PHEA | 1 mg/Kg | iv | Rats | liver | Spleen, lung, kidney, small intestine | Liver (faces excretion) Kidney (urine excretion) | t _{1/2} = 15-24 min (blood). t _{1/2} = 6-20 days (liver) | IONP labeled with 59Fe eliminated by kidney and faces IONP labeled with 14C eliminated by faces | Park2013 |
| Resovist 65 (hydro) | Dextran ± fucoidan | 2 mg/kg | iv | Mice bearing GL261 tumor | Liver, blood, spleen | Heart, kidney | Liver + kidney (w fucoidan) Liver (wo fucoidan) | t _{1/2} = 37 min w/o fucoidan t _{1/2} = 150 min w fucoidan | With fucoidan, two times more IONP in the tumor | Abdollah2018 |
| 18 | Rituximab | 6.4 mg/kg | iv | Mice bearing CD-20 tumor | Liver, spleen, kidney, blood, tumor, stomach (time dependent) % tumor = 9% ID/g. | Intestine, muscle | NA | NA | Compative kinetics for different organs: IONP in Lung, kidney, spleen, liver, intestine, stomach: ↗ during 5-15 h then ↘ IONP in blood, heart : gradual decrease IONP in tumor: gradual increase Accumulation in liver and spleen increases with increasing quantity of IONP administered | Azadbakht2018 |
| 10 (TEM) 47 (hydro) | Polyacrylic acid | 8, 20, 50 mg/kg | iv | Mice | Liver, spleen (dose dependant) | Lung, heart, tail | NA | NA | Accumulation in liver and spleen increases with increasing quantity of IONP administered | Couto2016 |
| 20 | PEGMA (LMW = 14 000 and HMW = 130 000) | 50 mg/Kg | iv | Mice | LMW: Spleen, liver, lung HMW: heart, lung, thymus, liver, spleen, kidney, tumor | LMW: Heart, thymus, kidney, tumor HMW: none | NA | t _{1/2} = 24h (HMW) t _{1/2} < 2h (LMW) | Much longer circulation time and higher accumulation in tumor for HMW than LMW | Ohno2013 |
| 26 (hydro) | fibrin | 5 mg/Kg | iv | Mice | Liver | Spleen, lung, kidney, heart | Urine and feces | t _{1/2} = 12 hours | Accumulation of IONP in liver and progressive departure of IONP from liver between 6h and 28d | Prabu2015 |
| 41 (hydro) | Dextran + DOTA | 4 mg/Kg | iv | Mice with breast tumor | Blood, liver, tumor | Kidney, stomach, intestine, colon, heart, lung, bone | NA | NA | Between 4h and 24h post inject., IONP leave blood and remain in liver and tumor % ID in tumor = 0.25 % | Rasaneh2015 |
| 12 | Glucose or PEG | 6 mg/kg | iv | Mice | Liver, spleen | NA | NA | NA | Accelerated degradation and clearance with PEG compared with glucose | Stepien2018 |

Table 2-2

EPR targeting of tumors with different types of iron oxide nanoparticle

| IONP type / size | Admin. Route | Quantity admin. | Tumor volume Tumor weight | Tumor type | Percentage of injected dose per gram of tumor | Percentage of IONP in tumor (% ID) | Ref. |
|---|--------------|-------------------|-------------------------------------|------------------------------|--|---|--------------|
| SPIO encapsulated in nanocapsule | iv | 2 mg (mice) | 100 mm ³ 0.2 g | CT-26 colon tumor | 2% | 0.2% | Al-Jamal2016 |
| G100 starch coated IONP (Chemicell) | iv | 1.7 mg (rats) | 50-70 mm ³ 0.1 g | 9L glioma | 5.10 ⁻³ % | 5.10 ⁻⁴ % | Chertok2007 |
| IONP of 30 nm | iv | 2 mg (mice) | 100-200 mm ³ 0.2 g | BT-474 breast cancer cells | 7% | 1.4% | Dong2015 |
| IONP of 100 nm | iv | 2 mg (mice) | 100-200 mm ³ 0.2 g | BT-474 breast cancer cells | 0.75-1.25% | 0.15-0.25% | Dong2015 |
| SPIO (6-10 nm) (Taiwan advanced Nanotech) | iv | 300 µg (rats) | 20 mm ³ 0.1 g | C6 glioma | 1% | 0.1 % | Fan2013 |
| SPIO with amino groups (36 nm) | iv | 4 mg (rats) | 23 mm ³ 0.1 g | C6 glioma | 0.1% | 0.01% | Fan2016 |
| SPIO embedded nanobubbles | iv | 3 mg (rats) | 100 mm ³ 0.2 g | Glioma tumor | 3% | 0.6% | Huang2013 |
| SPIO (10 nm) in nanocapsule | iv | 1.4 mg (mice) | 400-600 mm ³ 0.5 g | CT26 4T1 LLC B16F10 | 2.5% (CT26) 2% (4T1) 1% (LLC) 2% (B16F10) | 1.25% (CT26) 1% (4T1) 0.5% (LLC) 1% (BL6F10) | Mei2016 |
| SPIO (ZnMnFeO) | iv | 0.8 mg (mice) | 150 mm ³ 0.2 g | 4T1 tumor | 5% | 1% | Ni2018 |
| IONP (240 nm) IONP + Paclitaxel (240 nm) | iv | 0.2-0.5 mg (mice) | 50-100 mm ³ 0.1-0.2 g | CT26 | NA | 0.1% (SPIO) 0.1% (SPIO+ Paclitaxel) | Schleich2014 |
| SPIO + PEG (7 nm) SPIO + two PEG (7 nm) | iv | 0.125 mg (mice) | 100 mm ³ 0.2 g | 4T1 | 8% (IONP + PEG) 15% (IONP + 2 PEG) | 1.6% (IONP + PEG) 3% (IONP + 2 PEG) | Xu2016 |
| Starch coated D-MNP (Chemicell) conjugated with PEG and β-glucosidase | iv | 0.24 mg (mice) | 300-400 mm ³ 0.4 g | 9L glioma | 1% | 0.4% | Zhou2014 |
| Starch coated D-MNP (Chemicell) conjugated with β-glucosidase | iv | 0.24 mg (mice) | 300-400 mm ³ 0.4 g | 9L glioma | 0.5% | 0.2% | Zhou2014 |
| Starch coated D-MNP (Chemicell) | iv | 0.24 mg (mice) | 300-400 mm ³ 0.4 g | 9L glioma | 0.1% | 0.04% | Zhou2014 |

Table 3

Molecular targeting of tumors with different types of iron oxide nanoparticles

| IONP type ± targeting agent (TA) | Admin. Route | Quantity admin. | Molecular targeting (MDT) | Type of targeted tumor | Tumor volume/ Weight | Percentage of injected dose per gram of tumor (%ID/g of tumor) | Percentage of IONP in tumor (% ID) | Ref. |
|---|--------------|------------------|--|---|----------------------------------|--|---|---------------|
| SPIO (BNF) + PSMA antibody (with TA) | iv | 0.25-4 mg (mice) | PSMA antibody Targets PSMA | PC3 prostate tumor | 140 mm ³ 0.2 g | 2.9% (with TA) | 0.6% (with TA) | Azad2015 |
| SPIO (BNF) + anti-GD2 antibody (with TA) | iv | 0.073 mg (mice) | GD2 expressed on CHLA-20 neuroblastoma | CHLA-20 neuroblastoma Xenografts | NA | 5.7% (with TA) 0% (without TA) | NA | Baiu2015 |
| SPIO + Biotin (with TA) | iv | NA | Targets avidin injected in the tumor | NA | 130 mm ³ 0.2 g | 0.6% (with TA) | 0.1% (with TA) | Chauhan 2013a |
| SPIO + folate (with TA) | iv | NA | Targets folate receptors of KB cancer cells | KB cells | 1.6 cm ³ 1.9 g | 0.2-0.6% (with TA) | 0.38-1.14% (with TA) | Chauhan 2013b |
| IONP of 30 nm (without TA) IONP of 30 nm + Trastuzumab (with TA) | iv | 2 mg (mice) | Trastuzumab antibody binds to HER2 expressed on tumor cells | BT-474 breast cancer cells | 100-200 mm ³ 0.2 g | 7% (without TA) 15% (with TA) | 1.4% (without TA) 3% (with TA) | Dong 2015 |
| IONP of 100 nm (without TA) IONP of 100 nm + Trastuzumab (with TA) | iv | 2 mg (mice) | Trastuzumab antibody binds to HER2 expressed on tumor cells | BT-474 breast cancer cells | 100-200 mm ³ 0.2 g | 0.75-1.25% (without TA) 0.75-1.25% (with TA) | 0.15-0.25% (without TA) 0.15-0.25% (with TA) | Dong 2015 |
| IONP (without TA) IONP + Ffab (with TA) IONP + CMD + Ffab (with TA + CMD) | ip | 0.75 mg (mice) | Ffab binds to recombinant FOLRα (rFOLRα) | KB cells (human squamous cell carcinoma of the oral cavity) | NA | NA | 0% (without TA) 4% (with TA) 7% (with TA + CMD) | Ndong2015 |
| SPIO + human serum albumin (with TA) | iv | 0.12 mg (mice) | HAS may interact with gp60 and SPARC and yield improved extravasation and tumor accumulation | 4T1 breast tumor | 500 mm ³ 0.6 g | 2% (with TA) | 1.2% (with TA) | Quan2011 |
| SPIO + RGD (with TA) | iv | 0.2 mg (mice) | Targets α _v β ₃ integrin-expressing U87MG cells | Glioblastoma U87-MG | 180 mm ³ 0.2 g | 2.6-5.4% (with TA) | 0.6-1.2% (with TA) | Yang2011 |
| SPIO + PEG (without TA) SPIO + PEG + Trastuzumab (with TA) | iv | 0.5 mg (mice) | Trastuzumab antibody binds to HER2 expressed on tumor cells | SKBr3 breast cancer cells | 65 mm ³ 0.1 g | 0% (without TA) 8.8% (with TA) | 0% (without TA) 0.9% (with TA) | Zolata2015 |

Table 4

Magnetic targeting of tumors with different types of iron oxyde nanoparticle

| IONP type | Admin. Route | Quantity Admin. (animal) | Strength of magnetic field (with MT) | Type of targeted tumor | Tumor size Tumor weight | Percentage of IONP in tumor (% ID/g tissue) | Percentage of IONP in tumor (% ID) | Ref. |
|---|--------------|--------------------------|--------------------------------------|---------------------------|----------------------------------|---|---|--------------|
| SPION encapsulated in nanocapsule | iv | 2 mg (mice) | 0.43 T | CT-26 colon tumor | 100 mm ³ 0.2 g | 6% (with MT) 2% (without MT) | 0.6% (with MT) 0.2% (without MT) | Al-Jamal2016 |
| G100 starch coated IONP (Chemicell) | iv | 1.7 mg (rats) | 0.4 T | 9L glioma | 50-70 mm ³ 0.1 g | 5.10 ⁻² % (with MT) 5.10 ⁻³ % (without MT) | 5.10 ⁻³ % (with MT) 5.10 ⁻⁴ % (without MT) | Chertok2007 |
| G-PEI starch coated IONP (zeta potential = 37 mV, Chemicell) | ia | 1.7 mg (rats) | 0.35 T | 9L glioma | 70-90 mm ³ 0.2 g | 0.34 % (with MT) | 0.068 % (with MT) | Chertok2010 |
| G-100 starch coated IONP (Zeta potential = -12 mV, Chemicell) | ia | 1.7 mg (rats) | 0.35 T | 9L glioma | 70-90 mm ³ 0.2 g | 0.08%(with MT) | 0.16% (with MT) | Chertok2010 |
| SPION (6-10 nm) (Taiwan advanced Nanotech) | iv | 0.3 mg (rats) | 0.5 T | C6 glioma | 20 mm ³ 0.1 g | 5% (with MT + FUS) 2% (with MT) 1 % (without MT) | 0.5% (with MT + FUS) 0.2% (with MT) 0.1 % (without MT) | Fan2013 |
| SPION with amino groups (36 nm) | iv | 4 mg (rats) | 0.5 T | C6 glioma | 23 mm ³ 0.1 g | 0.5-0.8% (with MT) 0.1% (without MT) | 0.05-0.08% (with MT) 0.01% (without MT) | Fan2016 |
| SPION embedded nanobubbles | iv | 3 mg (rats) | 1.2 T | Glioma tumor | 100 mm ³ 0.2 g | 7% (with MT) 3% (without MT) | 1.4% (with MT) 0.6% (without MT) | Huang2013 |
| SPION (ZnMnFeO) | iv | 0.8 mg (mice) | Neodinium magnet | 4T1 tumor | 150 mm ³ 0.2 g | 12% (with MT) 5% (without MT) | 2.4% (with MT) 1% (without MT) | Ni2018 |
| MoS ₂ Fe ₃ O ₄ | iv | 0.4 mg (mice) | N.A. | PANC-1 (pancreatic tumor) | 100 mm ³ 0.2 g | 4% (with MT) | 0.8% (with MT) | Yu2015 |
| Starch coated D-MNP (Chemicell) conjugated with PEG and β-glucosidase | iv | 0.24 mg (mice) | 0.32 T | 9L glioma | 300-400 mm ³ 0.4 g | 6.5% (with MT) 1% (without MT) | 2.6% (with MT) 0.4% (without MT) | Zhou2014 |
| Starch coated D-MNP (Chemicell) conjugated with β-glucosidase | iv | 0.24 mg (mice) | 0.32 T | 9L glioma | 300-400 mm ³ 0.4 g | 3% (with MT) 0.5% (without MT) | 1.2% (with MT) 0.2% (without MT) | Zhou2014 |
| Starch coated D-MNP (Chemicell) | iv | 0.24 mg (mice) | 0.32 T | 9L glioma | 300-400 mm ³ 0.4 g | 1% (with MT) 0.1% (without MT) | 0.4% (with MT) 0.04% (without MT) | Zhou2014 |

Table 5

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