Pharmacokinetics variability: why nanoparticles are not magic bullets in oncology

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PHARMACOKINETICS VARIABILITY: WHY NANOPARTICLES ARE NOT MAGIC-BULLET IN ONCOLOGY

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Acknowledgments:

Anne Rodallec has a generous grant from the Ligue Contre le Cancer.

Abstract

Developing nanoparticles to improve the specificity of anticancer agents towards tumor tissues and to better control drug delivery is a rising strategy in oncology. An increasing number of forms (e.g., conjugated nanoparticles, liposomes, immunoliposomes…) are now made available on the shelves and numerous other scaffolds (e.g., dendrimeres, nanospheres, squalenes…) are currently at various stages of development. However, the attrition rate when developing nanoparticles is particularly high and several promising forms showing excellent behavior and efficacy in preclinical studies failed to succeed in subsequent first-in-man studies or later in phase-II trials. The issue of pharmacokinetic variability is a major, yet largely underestimated issue with nanoparticles. A wide variety of causes (e.g; tumor type and disease staging, comorbidities, patient's immune system) can explain this variability, which can in return impact negatively on pharmacodynamic endpoints such as lack of efficacy or severe toxicities. This review aims at covering the main causes for erratic pharmacokinetics observed with most nanoparticles. Should the main causes of such variability be identified, specific studies in non-clinical or clinical development stages could be undertaken using dedicated models (i.e., mechanistic or semi-mechanistic mathematical models such as PBPK approaches) to better describe nanoparticles pharmacokinetics and decipher PK/PD relationships. In addition, identifying relevant biomarkers or parameters likely to impact on nanoparticles pharmacokinetics would allow either modifying their characteristics to reduce the influence of the expected variability during development phases, or developing biomarker-based adaptive dosing strategies to

maintain an optimal efficacy/toxicity balance. Overall, we call of developing comprehensive distribution studies and state-of-the-art modeling support to help better picture and anticipate nanoparticles pharmacokinetics.

Key words: nanoparticles – pharmacokinetics –variability – liposomes – oncology – MPS – modeling.

Introduction:

In oncology, achieving and ensuring an optimal efficacy/toxicity balance is still a challenging issue. The narrow therapeutics margins of standard cytotoxics and the issue of low intratumor diffusion have triggered huge expectations from the development of nanoparticles [1]. Thus, if much kind of nanoparticles have been developed over the last decades, relatively few have been actually approved over the last years. However, a rising number of new entities are making their way from bench to bedside application (Table 1), displaying now a wide variety of forms and indications (Figure 1). Despite increasing efforts and resources in developing nanocarriers, little is known about their actual pharmacokinetics. Paradoxically, the expected higher therapeutic efficacy is mostly based upon improved pharmacokinetics (i.e., reduced clearance, higher specificity towards target organs) [1]. However, the few data made available regarding nanoparticles (i.e., liposomes) pharmacokinetics have reported higher interpatient variability as compared with standard drugs [2], as if behavior of nanoparticles in the body could be both more targeted and less predictable. Of note, interpatient variability in drugs exposures is a major cause for treatment failure in oncology, owing to the narrow therapeutic window of most anticancer agents [3]. To what extent this variability accounts for the particularly elevated attrition rates when developing nanoparticles remains to be fully elucidated. Here, this review will not exclusively but preferentially address the issue of liposomes pharmacokinetics in oncology, since most nanoparticles developed in cancer are liposomal drugs [4].

I. Expected improvements in pharmacokinetics with nanoparticles.

1. Achieving longer exposure through decreased clearance.

In vivo elimination of conventional liposomes and other nanoparticles has been extensively studied and reported before [6]. Briefly, it depends on upstream interactions with specific proteins in plasma then activity of the Mononuclear Phagocyte System (MPS) [6]. Macrophages play indeed a major role, as 80% to 90% of nanoparticles will get engulfed in the liver or the spleen to get degraded. Although this process occurs rapidly, liposomes shows longer stay in the body as compared with free drugs [7]. Even if first-generation nanoparticles displayed reduced clearance as compared with standard drugs, different strategies have been further developed next to limit organ uptake and immune system-related clearance. The most common strategy consists in masking the nanoparticle through surface pegylation, thus generating stealth, or second-generation nanoparticles [1]. Second-generation nanoparticles are less likely to be recognized by MPS and accumulate in the spleen and the liver [8], enabling the drug to stay longer in the blood stream, as demonstrated for instance for stealth liposomal doxorubicine [9].

2. Reducing toxicity via higher tumor specificity

2.1 Passive tumor targeting: the EPR effect

Solids tumors present a leaky vasculature originally allowing nutrient supply, necessary for sustained tumor growth. This anarchical organization has been defined by Maeda as enabling the Enhanced Permeability and Retention (EPR) effect [10]. Nanoparticles

could passively target the tumor by going through the vasculature gaps (i.e., 200 nm [11]) to be retained near the tumor because of deficient lymphatic drain (Figure 2). Radiotherapy has been sometimes used to enlarge these gaps by depleting the pericytes, so as to further enhance permeation [12] and thus tumor accumulation [13]. Developing stealth agents (e.g. PEG, see above) are other strategies to increase the EPR effect since the longer nanoparticles stay in the blood, the more they will pass through the vasculature gaps to target tumor tissues. This could explain why some nanoparticles display both decreased clearance and higher volume of distribution (Vd), with a limited drug accumulation in healthy tissues [1].

2.2 Active tumor targeting

In spite of a more specific delivery to the tumor, the EPR effect alone usually achieves less than a 2-fold increase [14] of tumor accumulation. Efforts have thus been made for developing third generation nanoparticles that display more active targeting. This is mostly achieved by grafting on the surface of the carrier an agent that will recognize specifically cancer cells (Figure 3). Many moieties (e.g., small-molecule ligands, peptides and monoclonal antibodies) have been used over the last years to actively target cancer cells through different strategies, including targeting EGFR [15], folate and transferrin receptors, tumor antigens [16] and neo-antigens showing on the surface of irradiated tumor cells [17]. Bioengineered-albumin can also be used as a targeting agent, by generating albumin-bound nanoparticles. So far, albumin-bound paclitaxel is the only example of such scaffold (nab-paclitaxel, ABI-007) that showed better performances in terms of tumor tissue/healthy tissue balance as compared with free

paclitaxel [18]. Such targeting is achieved because nano-albumin has a higher affinity for SPARC glycoproteins that is found overexpressed in many cancer types such as breast or pancreatic cancers. Folic acid can also be used as a targeting agent, because it is recognized by folate receptors which are overexpressed in many cancer cells, thus ensuring eventually a better trafficking into tumors [19].

II. Predicting nanocarrier pharmacokinetics: an ongoing challenge.

1. How to anticipate Carrier-driven Pharmacokinetics

1.1 Size.

As discussed since 1999 [20], size is a major factor not only for the residence time of nanoparticles in blood but also for further tumor targeting performances. The smaller is a nanoparticle the less it will be recognized by the MPS and eliminated from the body [21]. However it has been demonstrated that nanoparticles <8 nm are mostly eliminated by the kidneys [22], not to mention loss of stability in plasma and therefore quicker clearance below a given size. Being too big (i.e., > 200 nm) is also a major drawback, since it prevents nanoparticles to benefit from the EPR Effect. Several studies have shown how size can affect the distribution phase within the tumor tissues and does matter indeed for tumor accumulation. When testing three different batches of stealth liposomes of 5-FU varying in size (i.e., 70 to 250 nm) in mice bearing resistant breast tumor, data showed that the smaller the liposomes, the greater the tumor uptake [23]. Consequently, better efficacy and longer survival were achieved in animals treated with smaller liposomes, thus demonstrating how size can impact on tumor tissue distribution

and efficacy endpoints eventually. Similar results were found by Charrois et al. who studied the influence of liposomes diameter on tumor distribution in mice bearing mammary carcinoma [24]. Statistically significant lower accumulation and reduced efficacy were evidenced for bigger liposomes. Overall, those experimental results confirmed older studies reporting that the optimal size for nanoparticles has to be in the 100-200 nm range, much probably because of the EPR effect [25].

1.2 Composition

The use of stealth or targeting agent deeply modifies the drug pharmacokinetics. In addition, the choice of components is a key factor too since it will modulate the stability of the nanoparticle in the systemic circulation by affecting the RES recognition and subsequently the drug release. Unstable nanoparticles will display increased plasma clearance and reduced circulating times, as compared with stable nanoparticles. The Gregoriadis group extensively studied the major role of the composition in phospholipids and cholesterol in the early 1980's [26]; subsequent studies have further addressed the issue of lipids and cholesterol ratios required to achieve the most stable liposomes with optimal controlled release, especially the critical role cholesterol plays [27]. Indeed, cholesterol's inclusion in the lipids bilayer of a liposome stabilizes its structure and decreases drug leakage and risk of opsonisation, thus extending circulation time [5].

1.3 Electric Charge

The zeta potential of nanoparticles is another major factor influencing stability and pharmacokinetics. Of note, this potential depends all on the components used to

synthesize the nanoparticle. Geng S et al. studied the impact of cholesterol on a doxorubicin PEGylated liposome stability, but this time focusing on electric charge [28]. Using two cholesterol derivatives (i.e., positively charged VS. neutral), pharmacokinetic studies in rats showed that neutral cholesterol liposomes achieved higher stability than positively charged ones. Gabizon et al. tested in mice the impact of negatively charged lipids on liposomes clearance and found that it depended on their composition [29]. Similarly, the Torchillin group evaluated in mice the clearance of liposomes displaying different surface properties [30]. Different charged lipids were tested, with or without surface pegylation. Charged liposomes showed higher clearance, especially the negatively charged one (i.e., with PA or PS) which were preferentially found in the liver. Adding PEG-750 helped counter-balancing the higher clearance of positively (i.e. with SA) charged liposome but no that of negatively charged ones. Conversely, PEG-5000 partly reduced negatively charged liposome clearance, thus highlighting how complicated can be the combined impacts of electric charge and pegylation on subsequent nanoparticles pharmacokinetics. Recent studies have confirmed the deleterious impact of the negative charge on liposome clearance, and showed how pegylation can help improving their pharmacokinetics [31]. Additionally, other studies focused on the impact of positively charged nanoparticles on tumor uptake. For instance, Campbell and al. have studied the biodistribution of cationic liposomes in human colon cancer bearing mice [32]. The impact of cationic lipid ratio on distribution was investigated: increasing cationic lipid by 10% decreased spleen uptake, whereas further increase did not reduce anymore liposome accumulation in spleen. Regarding tumor uptake, although differences in the total tumor accumulation were not statistically

significant, intravital microscopy revealed that cationic charges specifically target tumor vasculature. Increasing the charge content led to doubling the neo-vessels uptake, suggesting its impact on the tumor distribution and the benefit of using charged lipids for further increasing tumor specificity. Indeed, as compared with neutral liposomes, the cationic ones display a higher tumor uptake that can be hindered when pegylated. Positive charge and pegylation are then two opposite characteristics that can modulate tumor specificity. Both can be combined to achieve adequate targeting. For instance, Li and al. evaluated the quantitative relationship between these parameters on pancreatic cancer cells [33], using liposomes with alterable zeta potential and using a methoxy-analog of PEG-DSPE to reduce the electric charge. Data showed that each mol % of PEG could be compensated with a 4 mV increase, thus suggesting the existence of a balance between those two parameters to maximize the stealth property while ensuring tumor internalization of cationic liposomes. Once again, this highlights the complexity of how carrier composition must be finely tuned to optimize its pharmacokinetics eventually, especially at the tumor level.

1.4 Shape

Although most of the developed carriers are spherical, other shaped nanoparticles showed interesting properties [34]. Shape is another major parameter indeed related to macrophages uptake and subsequent nanodrug biodistribution [35]. Macrophage-induced phagocytosis and therefore clearance not only depends on the size but also the geometry of the particle. Champion et al. described the behavior of macrophages against six nanoparticles shaped differently [36]. Spheres, oblate ellipsoids, prolate

ellipsoids, elliptical disks, rectangular disks, and UFOs shapes were studied. It was observed that the initial angle contact between the macrophages and the nanoparticles influences its phagocytosis and therefore its clearance. For instance, phagocytosis of elliptical disks could be 20 fold longer if macrophages attached along their minor axis, thus illustrating how shape is critical to reduce recognition by macrophages. Similarly, Barua et al., evaluated the impact of particle shape on its tumor internalization [37]. They studied tumor uptake of spherical, rod- and disk-shaped particles in different breast cancer models. When uncoated, spheroid nanoparticles showed higher tumor specificity. However, those results were different when adding trastuzumab as targeting agent. Indeed, when coated, tumor internalization of rod-shaped, and to a lesser extent disk-shaped particles, were higher than with the spherical ones.

1.5 Protein corona

When in biological fluids, the nanoparticle surface attracts proteins and biomolecules which will form around the liposome a dynamic layer, exchanging continuously with the environment. This rapidly shaped layer is usually called protein corona. Its composition depends on the surface properties of the nanoparticle, the nature of the environment, the time of exposure and the tumor type [38]. The protein corona can affect liposome specificity by modifying its surface properties (i.e., charge, size) or by hampering targeting agents [39]. However, the presence of specific proteins in the corona can also improve tumor uptake. Corbo and al. demonstrated that the presence of apolipoproteins and immunoglobulins in the corona increased liposome uptake in breast cancer cells [40]. They found however a similar increase in macrophages uptake, modifying the drug

release rate from the carrier. When engulfed by the MPS, the encapsulated drug is quickly released indeed and stops benefiting from advantages such as a longer half-life and a more specific biodistribution towards tumor tissues. Thus, efficacy/toxicity balance can be affected [41], because the nanoparticle pharmacokinetics becomes quickly similar to that of the free drug.

2. Separating the free drug from the carried one: an ongoing challenge.

The lack of comprehensive pharmacokinetic studies with nanoparticles can be partly explained by the fact that it remains difficult to discriminate carried drugs from free drugs in the blood stream. Depending on the nanoparticle stability and its pharmacokinetics, part of the payload can be released early from the carrier. Consequently, both carried drug and free form are found together in plasma, but most standard bioanalytical techniques used when performing PK studies will fail in discriminating them. Direct methods usually used to differentiate non-protein-bound drugs to bound-ones, like solid-phase micro extraction could be adapted to measure the amount of drug remaining inside the liposome [42]. For instance, Hempel's group developed an analytical method based on solid-phase extraction followed by capillary electrophoresis with fluorescence detection, to quantify drug concentration of free and encapsulated daunorubicin in plasma [43]. Another promising technique is based upon microdialysis probes applied to nanomedicine. Microdialysis technique relies indeed on the passive uptake of free, unbound drug only. If the cut-off of the semi-permeable membrane is smaller the nanoparticle diameter, then only the free released drug will cross the membrane to be further analyzed. Parallel blood sampling will allow measuring the whole drug, thus enabling to discriminate encapsulated and non-

encapsulated drug. Of note, only the free-fraction of the released drug will be measured; however, by knowing the protein binding of the drug it is possible to calculate the whole concentration eventually. In those conditions, Zamboni's group was able to measure *in vivo* the percentage of encapsulated and released platinum and topotecan [45]. The released drug displays theoretically the same pharmacokinetic parameters than the standard, non-encapsulated form. However, because its own release rate is closely related to the pharmacokinetics of the carrier, its ADME profile depends eventually on the upstream behavior of the carrier in the body, thus generating an erratic behavior because its Ka is likely to be impacted by the pharmacokinetics of the carrier. Indeed, the absorption rate Ka of the released drug directly depends on the Ke elimination rate of the nanoparticle, which itself is related to the stability of the carrier (see above). Drug release varies therefore upon the nanoparticle composition, the fabrication process and the drug properties (Figure 4). Nounou et al. used compared in vitro the release rates from a liposomal hydrophobic drug (i.e., dibucaine base) and a more hydrophilic drug (i.e., 5-fluorouracil) [46]. Results showed major differences between the two drugs. Whereas liposomal dibucaine was stable, 5-fluorouracil nanoparticles showed a burst effect, with a fast and early leakage followed by a constant release phase. Studying drug release keeps being improved with the recent development of numerical deconvolutions that can evaluate the drug release directly from the nanoparticle, and no longer from the receiver compartment [47].

3. How tumor characteristics can affect nanoparticle pharmacokinetics?

3.1.1 Size and Vasculature of the tumor

It has to be underlined that the disease can affect nanoparticle pharmacokinetics, for instance by varying in neo-vessels density depending on tumor burden or cancer type. To evaluate the impact of the tumor itself on the EPR effect, Hirsjärvi and al. studied *in vivo* the biodistribution of a 50 nm nanocarrier on four different tumor types (i.e., glioblastoma, breast cancer and two liver cancer models) [48]. Results showed a similar distribution profile in healthy tissues (i.e., heart, lung, brain, skin, muscle, kidney, bladder, intestine, spleen, pancreas, fat, stomach, liver and lymph node) but a marked heterogeneity regarding tumor delivery, depending on the cancer type. Accumulation was much higher indeed for glioblastoma and breast cancer cells. Therefore, the two liver cancer models were classified as “weak EPR effect” tumors, illustrating how tumor characteristics should be a new parameter to consider for understanding and predicting the pharmacokinetics of nanoparticles at the tumor level. This heterogeneity in EPR effect found by Hirsjärvi and al. between tumors can be related to the variability in tumor growth. Liver cancer models showed indeed a slower growth than glioblastoma and breast cancer models, as a result of reduced neo-vascularization [48]. Fanciullino et al. confirmed in breast cancer bearing mice that vascular density was a key-factor impacting on the extent of the tumor uptake of stealth liposomal 5-FU uptake measured at different cancer stages [23]. A strong correlation was evidenced between tumor size, vascular density and the accumulation of liposomes, suggesting a lower accumulation in metastasis. Although only experimental, such data provided clues for better understanding the negative impact anti-angiogenics drug could have when combined with nanoparticles as reported in phase II trial combining bevacizumab and nab-paclitaxel [49]. It is however suggested that bevacizumab induces a transitory vascular

normalizing effect on tumor neo-vessels [50], and that during this phase of normalization the amount of associated cytotoxic reaching the tumor would be greater [51]. Therefore, sequential administration of antiangiogenics followed by nanoparticles could improve the efficacy of the combination. In this context, mathematical models could be used to predict the vasculature normalization [52] so as to determine the best time-window to optimize the distribution of nanoparticles when combined to anti VEGF agents.

3.1.2 Tumor Microenvironment

The tumor microenvironment including all the non-neoplastic cells such as fibroblasts, immune cells, stem cells, and endothelial cells [53] could modulate the EPR effect [54]. Zamboni's group studied in mice bearing melanoma and ovarian xenografts the relationships between tumor accumulation of pegylated-liposomal drug and MPS [55]. The greater presence of macrophages and dendritic cells in the tumor extracellular fluid of ovarian model was related to increased tumor delivery of pegylated liposome when compared to melanoma model. These results suggest the close relationship between nanoparticle delivery and the MPS. More recently, the same group confirmed the importance of tumor microenvironment heterogeneity depending on tumor type, by profiling the MPS in mice bearing ovarian, breast, and endometrial xenografts [56]. Macrophages were quantified in the liver, the spleen and the tumor and marked differences were found between the tumor types. Significantly more macrophages were found in both liver and spleen in animals with endometrial cancer than those with breast and ovarian cancers. Similarly, differences in tumors infiltrating macrophages depending on cancer types were observed. Significantly more macrophages were found

in breast cancer models as compared with ovarian and endometrial tumors. This macrophage disparity in the liver, the spleen and the tumor was also reported for various cell lines within a same tumor type. Because macrophages play an important role in the clearance of nanoparticles, such variability can impact their pharmacokinetics at the tumor level, thus explaining why and how tumor type could affect nanoparticles pharmacokinetics. In addition, because tumor microenvironment can be considered as a target related to tumor growth and to drug delivery, several studies have focused on strategies to affect its barrier. Chen and al. oxygenated the tumor microenvironment to decrease head-and-neck cancer resistance to radiations and chemotherapies [57] whereas Hingorani and al. proved that pegylated hyaluronidase (PEGPH20) can be useful to enhance the efficacy of nanoparticles. Hyaluronic acid halos in the tumor micro-environment matrix reduce tumor perfusion and therefore limit the access of nanoparticles. In a recent phase-II trial, PEGPH20 was associated to nab-paclitaxel plus gemcitabine in pancreatic cancer. Both responses and survival were improved using this strategy [58]. Finally, triggered drug delivery systems are a promising option to release the drug from the nanocarrier in tumor surroundings only. It can be functionalized with heat, ultrasound, light, enzymes or pH [59]. Ph-triggered nanoparticles such as liposomes and micelles can specifically release their bioactive content in the tumor microenvironment, because of the acidic tumor surroundings [60], highlighting how tumor specificities can be used as a Trojan horse to target cancer tissues.

III. Main Causes for NPs pharmacokinetic variability

1. Age

Because nanoparticles, especially the stealth ones, are expected to bypass liver uptake and to avoid renal elimination, age which affects usually those organs should theoretically not be a factor of variation (Fig.5). However, because age affects the MPS, it can in some respect change nanoparticles pharmacokinetics [61]. For instance, pharmacokinetics of doxorubicin encapsulated in PEGylated liposomes was studied in 35 elderly patients. Doxorubicin plasma levels, leukocyte DNA breaks and monocytes count variations were measured as PK and PD endpoints. Results showed a 30% increase in mean T1/2 with two-fold increase in drug plasma levels for patients > 77 years old. Older patients (i.e., > 80 years) showed even more extended half-life [62]. In addition, age can also be associated with increased toxicities upon nanoparticles administration. Wu and al. also related age-effect with irinotecan PEGylated liposome (IHL-305) in patients with advanced solid tumors [63]. Neutrophils and monocytes decrease were found lower in younger patients (i.e., <60 years old), with an inverse correlation between monocytes decrease and clearance of total irinotecan. Consequently, it seems that nanoparticles may display reduced clearance and subsequent increased exposure and pharmacodynamic effects (efficacy, toxicity) in the elderly, thus advocating for adaptive dosing strategies in older patients.

2. Body mass

Body mass is usually best described through BMI or body surface area (BSA). It can affect drug pharmacokinetics because the Vd depends partly on hydrophilic or lipophilic profile (i.e., lipophilic drugs tend to accumulate in fat tissues). Considering the more

specific distribution achieved with nanoparticles, limited impact is expected from changes in body mass. However it has been suggested that body weight could modulate the clearance of nanoparticles via changes in the MPS [64]. In a clinical study, LaBeck and al. evaluated factors that affect pharmacokinetics of PEGylated liposomal doxorubicin as part of phase I/II studies in 70 patients with either solid tumors or Kaposi's sarcoma. BSA and BMI were both evaluated as potential covariates in the clearance values. BSA, but not BMI, contributed to a non-significant reduction in the clearance variability, thus suggesting that its impact was only marginal. However, Wu and. al showed that clearance of pegylated liposomal irinotecan (IHL-305) was dependent on a composite marker between body mass and age [63]. The relationship between the ratio of total body weight to ideal body weight (TBW/IBW) and age with IHL-305 pharmacokinetics was evaluated, and showed that old patients plus TBW/IBW higher than the median displayed higher levels of free circulating irinotecan, thus suggesting a loss of stability of the nanoparticle in elderly patients with overweight. Increased proteinemia in overweight people could explain this difference, and suggest that specific populations (e.g., obese patients) should have their dosing tailored.

3. Gender

It is well established that gender is a factor affecting a drug pharmacokinetics, distribution and clearance because of sex differences in body mass distribution, enzymes activities, to name but a few [65]. La Beck and colleagues evaluated using non-compartmental methods the impact of gender on the clearance of three PEGylated liposomes. For each nanocarrier, female patients displayed lower clearances as

compared with males, a finding consistent with previous reports regarding gender difference in drug pharmacokinetics [66]. With nanoparticles, the most significant results were found for doxorubicin PEGylated liposome because female patients showed a 58% reduction in clearance as compared with men [66].

4. Drug-drug interactions

Only few studies have reported drug-drug interactions with nanoparticles. Because of reduced liver uptake and the fact that nanoparticles are not substrate of efflux transporters, there are less likely to be affected by inhibiting/inducting drugs. However, liposomal doxorubicin (Doxil®) showed higher AUC and decreased clearance when co-administrated with paclitaxel or docetaxel [67]. Inversely when given after Cisplatin, the clearance of Doxil® was increased, although no clear underlying mechanisms has been identified yet [68]. Other clinical data suggested possible interaction of liposomal doxorubicin with bevacizumab. When given as a combination for treating locally recurrent or metastatic breast cancer, they triggered more toxicities than expected, suggesting overexposure [68]. As related earlier, bevacizumab and to a broader extent anti-angiogenics are also suspected to decrease nanoparticles efficacy through a diminished EPR effect, although no experimental data have demonstrated this point [49].

5. Immunity

Nanoparticle clearance being partly controlled by the immune system and more specifically the MPS, evaluating its activity and possible causes for variations is critical [69]. A preclinical study demonstrated the influence of the tumor type on the MPS and

resulting impact on nanoparticles clearance [70]. The modification in immune cells population near the tumor is indeed well established [71], and can affect the MPS global activity. Decreased circulation time of the nanoparticle was observed in tumor-bearing mice as compared with healthy mice and among the different xenografted cancer models. Such discrepancy was explained by an increase in M2-like macrophages activity, demonstrating the importance of tumor type on the variability of the MPS activity. Prior treatment, especially with cytotoxics, is another major factor explaining variability in MPS activity. Most cytotoxics will indeed affect the MPS and subsequently the nanoparticles clearance. For instance, in a phase-I study of liposomal camptothecin analog, deep and equal decrease in both monocytes and neutrophils was first found with standard CKD-602 [72]. Conversely with liposomal S-CKD602, decrease in monocytes was deeper than in neutrophils. This discrepancy could be explained by the fact that liposomes are likely to be engulfed in monocytes. Gusella and al. also demonstrated the major effect of previous treatment on doxorubicin pegylated liposomes administrated in patients above 70 years old [62]. They found a reduction in nanoparticle clearance throughout cycles, related to monocyte count, suggesting that treatment-related impact on the MPS will, in return, modify the pharmacokinetics.

6. Genetic polymorphism

Germinal polymorphisms affecting genes coding for proteins implicated into ADME (liver enzymes, membrane transporters) can be major causes for pharmacokinetic variability with anticancer agents. For instance, genotyping of UGT1A1 (UDP glucoronosyltransferase 1A1) allelic variants (i.e., *UGT1A1*28*) is recommended when giving irinotecan to colorectal cancer patients [73]. UGT1A1 being involved in active

SN38 elimination, poor-metabolizer (PM) patients bearing the *UGT1A1*28* variant could experience severe hematological toxicities. Reduced hepatic clearance for liposomal drugs may lower the role of the liver and the relevance of *UGT1A1* genotyping when liposomal irinotecan or SN-38 are administered. However, a phase-I pharmacokinetics study on IHL-305 liposomal irinotecan in advanced solid tumor patients was performed with pharmacogenetic support. Two subsets were evaluated: patients with wild-type (wt) allele of UGT1A1 gene and patients with the *UGT1A1*28* homozygous variant. Results showed that patients with the homozygous *UGT1A1*28* variant could be safely administered with the nanoparticle provided that 50% reduction in dosing was performed [74]. Similarly, when developing liposomal irinotecan MM-398 (Onyvide®), starting with half-dose for the first course was recommended in *UGT1A1*28* patients, then switching to standard dose next if the first administration was well tolerated. More recently, a phase-1 study of liposomal SN-38 also demonstrated a good safety profile, regardless of the *UGT1A1* genotypes [75]. Similarly, *DPYD* genetic polymorphism leading to DPD deficiency syndrome strongly impact on the PK and safety of standard 5-FU, one of the most widely prescribed anticancer agent. In a non-clinical study in rodents, it has been demonstrated that stealth liposomal 5-FU was only moderately affected by the DPD status (i.e., DPD deficiency) in terms of pharmacokinetics and toxicities, whereas standard 5-FU administered to DPD-deficient rats led to sharp plasma overexposure and subsequent severe neutropenia [76]. This illustrates how developing stealth nanoparticles to bypass at least partly liver uptake and metabolic clearance could help reducing the deleterious impact of genetic polymorphisms affecting liver enzymes. Finally, the role of genetic factors on doxorubicin PEGylated

liposome was recently studied in mice. Results showed a correlation between nanoparticles clearance with a variation within a genomic region encoding for Gulp 1, a protein necessary for the engulfment of apoptotic cells by phagocytes. This suggests new genetic variants potentially involved in inter-patient variability observed with nanoparticles [77].

IV. Mathematical models: a tool for a better understanding of nanoparticles pharmacokinetics?

1. Mechanistic models of nanodrugs distribution

At the scale of a tumor and its associated vasculature, mathematical models can be derived for the intra-tumor drug transport and have relevance to address important questions for the design of nanoparticles. First, static differences in the fractal organization of the vasculature between tumor and healthy tissue have been evidenced and characterized by mathematical constructs [78]. Going further, biophysical models attached to the description of interstitial tumor drug transport have been developed [79]. These are based on established biophysical laws such as Starling law for flow across semi-permeable membranes, Darcy's law for flow through porous media or Poiseuille's equation for fluid velocity profiles [72]. Therefore, they rely on biophysically meaningful and measurable parameters which in turn allows to make quantitative predictions. Liu et al. reviewed computational techniques for modeling of nanomedicine, including continuum and stochastic-based methods [80]. The interest of such techniques lies in

their ability to predict (and thus, optimize) the outcome on tumor cell kill of specific characteristics of nano-carriers such as their size, drug release rate or binding affinity. They rely on the derivation of equations that allow the computation of quantities of fundamental importance for drug transport such as the blood vessels velocity profile, the interstitial fluid pressure and the encapsulated or free drug concentrations in the tissues. Comparative effects of diffusive and convective transport can then be computed, leading to quantitative predictions of the amount of drug effectively reaching the tumor cells and ultimately killing them, as a function of the nanocarrier properties (such as its size, weight, shape, drug load or drug release rate). For instance, the impact of multi-stage release of nested nanoparticles has been computationally investigated in where simulations were used to tune drug release kinetics and binding affinities in order to improve the drug delivery, and favored smaller nanoparticles. Similar investigation of multi-stage gold nanoparticles was also performed in [81]. In another study from the same group, modeling was employed to investigate the impact of post-angiogenesis inhibition vascular normalization [82] on the delivery of nanoparticles and demonstrated that drug delivery was improved but only for small (i.e., 12 nm diameter) nanoparticles [83]. One level of complexity higher, other groups have developed models still based on the principles of continuum mechanics that integrate two additional components: tumor cells (possibly composed of several phases) and angiogenesis. Indeed, the 1990's have witnessed extensive mathematical modeling research for description of spatially distributed tumor growth, with various levels of complexity and phenomena taken into account at the tumor scale (e.g., avascular versus vascular tumor growth, hypoxia, necrosis, invasion and interactions with the extra-cellular matrix) [84]. A

dynamic interplay exists between tumor growth and the development of the surrounding vasculature (necessary to grow beyond the diffusion limit of nutrients, i.e. a few millimeters in diameter), which motivated mathematicians to develop even more complex models of vascular tumor growth [85]. These have relevance in the context of nanoparticles delivery. Indeed, when coupled with hydrostatic laws of network fluid distributions, the models can predict perfusion features of the tumor vasculature and associated heterogeneity [81]. However, due to the technical difficulty to obtain morphological data of blood networks formation and blood flow in vivo, it is very challenging to validate them against experimental data [86]. Building on a model that incorporates the above-mentioned features [87], the first model integrating tumor growth with delivery of nanoparticles compared the delivery of a cytotoxic drug either via free drug administration or via 100 nm nanocarriers [88]. They found that drug transport limitations were severe, with important areas of the tumor where the drug concentration did not reach adequate levels. In a subsequent study, quantitative data from intravital microscopy was combined to computational simulations to determine how much drug per particle and how many particles need to be released in the vasculature to ensure tumor decay [89].

2. Pharmacokinetics (PK) / Pharmacodynamic (PD) and Physiologically-based pharmacokinetics models to better understand nanoparticles pharmacokinetics.

PK models are usually divided into compartmental versus non-compartmental approaches. The compartmental approach consists in an abstract representation of the

body as divided into compartments. Mass balance laws for transfers of the drugs between the compartments are then applied and formalized as ordinary differential equations. Non-compartmental approaches on the other hand directly describe the drug concentration as a function of time, without deriving this expression from any representation of the processes at play. Both approaches are attached to the characterization of the kinetics and exposure of the drug, as a function of fundamental parameters such as the clearance. A particularly interesting and useful tool is the population approach in which, using nonlinear statistical mixed-effects models, description of the inter-patient variability of the model parameters and associated quantities of interest can be quantified. Moreover, covariate analysis allows identifying meaningful subgroups that respond differently as a function of their sex, age or any other clinical feature such as genetic polymorphism or data regarding MPS status in cancer patients. While widely spread in the area of small drug pharmacokinetics, such modeling remains limited for nanodrugs. In [90], Wu et al. used such a population approach to characterize the pharmacokinetics of a PEGylated liposomal formulation of irinotecan and considered the distribution of the encapsulated, released and metabolized forms of the drug. Their results emphasized gender as an important covariate, as seen above (see also [91] for a similar study with another anticancer liposomal-encapsulated drug). For determination of the initial dose in Phase-I clinical trials, it is essential to be able to predict PK disposition in humans from animal data. Classical approaches for PK interspecies extrapolation consist mainly in allometric scaling laws (with body weight or other physiologically relevant variable). A study of the applicability of allometry was performed to determine the clearance in humans of

several pegylated liposomal and nanoparticle anticancer agents [92]. Due to the particularity of the elimination process for these molecules (through the mononuclear phagocytic system rather than kidney and/or liver, see above), the authors integrated to their analysis variables potentially related to this process such as the spleen weight or the total monocyte count. Nevertheless, an important discrepancy between the clearance predictions and observations from a Phase-I clinical trial was obtained, possibly because of the influence of tumor type. To perform interspecies scaling in more details, PBPK models have recently been developed for the PK of nanoparticles (Figure 6) [93]. These are based on a more realistic and intricate description of the vascular system and organs vascular distribution than with mere aggregated compartments. Lin et al. used a PBPK model for gold nanoparticles first developed in mice [94] and further extended to other species including human [95]. Based on a common structure for all species and species-specific adaptation of physiological parameters (thus determined *a priori*), they obtained good predictions of concentrations independently measured in humans. Together, mathematical modeling offers a powerful comprehensive framework for interpretation/analysis of experimental data at various scales which provides quantitative information and predictions useful for the optimal design of the nanoparticle itself (tumor-scale models) and determination of improved scheduling strategies (dosing and timing) both for clinical trials and personalized clinical routine (organism-scale PK models).

Conclusion & Perspectives: nanoparticles are not magic bullets!

Far from being the universal “magic bullet” once expected, the nanoparticles show a wide range of different parameters possibly impacting on their pharmacokinetics, and therefore their efficacy/toxicity balance. When studying the pharmacokinetics of nanocarriers, one must consider three distinct pharmacokinetic profiles: the systemic one, the tumor microenvironment one and the tumor one. In addition, discriminating free released drug and drug encapsulated or conjugated to its carrier is critical. This singularity plus the wide variety of factors possibly impacting on the ADME process may contribute to the greater pharmacokinetic variability described with liposomes by Schell and colleagues [2]. Of note as previously mentioned, carried drugs are expected to stay in the body much longer than standard drugs and sampling plan to perform PK studies with nanoparticles should probably be adapted to this new profile, rather than being based on standard sampling times that could be less informative. Moreover, patients involved in most Phase-1 studies generally present a wide range of different solid tumors. As discussed previously, characteristics inherent to tumor-type (i.e. size, vascular density, tumor micro-environment) are likely to change the pharmacokinetics of nanoparticles, thus adding again to the global inter-patient variability. Overall, a better and more comprehensive understanding of the specificities in nanoparticles pharmacokinetics could help selecting tumors the more likely to benefit from a nanocarrier. To achieve this goal, *in vitro* techniques more representative of tumor structure and tumor microenvironment, are currently being developed. For instance, co-culture models emerged by adding fibroblasts and other cell types, thus better mimicking components of the tumor microenvironment [96]. Another emerging model with nanoparticles is working with 3-dimentional (3D) tumor spheroids [97] as the issue

of tumor uptake cannot be properly described in standard 2D monolayer models [88]. More sophisticated models are now developed, such as those combining co-culture with 3D model [98] or developing patients-derived organoids, all being even closer to the *in vivo* representation of liposomal accumulation and drug release in the tumor. Similarly, *in vivo* techniques are being improved, with the development of genetically engineered cancer models that can address better than canonical xenograft models, tumor heterogeneity to correctly predict the pharmacokinetic parameters [99]. Of note, with regard to the important role played by the immune system on nanoparticle pharmacokinetics, switching from immune-compromised mice to syngeneic models could probably help to better picture the actual PK profile of nanocarriers during non-clinical development phases. Finally, owing to the complexity in picturing the whole pharmacokinetics of most nanoparticles, developing and using sophisticated models derived from applied mathematics is a critical, yet largely underestimated tool. Although limited, when available model-driven studies (i.e., using PB-PK approaches) seem to perform better than trial-and-error studies, thus possibly reducing the high attrition rates observed when developing nanoparticles and bridging the gap between bench and bedside.

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Table:

Table 1: Examples of clinically approved nanomedicines

Product name	Manufacturer	Description	Targeted cancer	Approval details
Oncaspar	Sigma tau pharmaceuticals Inc.	PEGasparaginase	Accute lymphoblastic leukemia	1994
Doxil	Centocor Ortho Biotech, J&J	PEGylated Doxorubicin liposomes	Recurrent ovarian cancer, AIDS-related Kaposi sarcoma, Multiple myeloma	1995; EMA for metastatic breast cancer (Caelyx)
DaunoXome	Galen, Ltd.	Daunorubicin liposomes	HIV-associated Kaposi Sarcoma	1996
DepoCyt	Sigma-Tau Pharmaceutical, Inc.	Cytarabine liposomes	Lymphomatous meningitis	1999
Myocet	Sopherion Therapeutics, LLC and Cephalon, Inc.	Doxorubicin liposomes	Metastatic breast cancer	2000 in Canada and Europe
Neulasta	Amgen, Inc.	PEGfilgrastim	Chemotherapy-associated neutropenia	2002
Abraxane	Celgene	Albumin bound - paclitaxel	Metastatic breast cancer, advanced	2005, 2012, 2013

			NSCLC, late stage pancreatic cancer	
Marqibo	Talon Therapeutics Inc.	Vincristin liposomes	Philadephia chromosome-negative acute lymphoblastic leukemia	2012
Kadcyla	Genentech, Inc.	Ado- Trastuzumab Emtansine	Recurrent HER2-positive, metastatic breast cancer	2013
Onivyde	Merrimack Pharmaceuticals, Inc.	PEGylated irinotecan liposome	Advanced (metastatic) pancreatic cancer	2015
Paclical	Oasmia Pharmaceutical AB	Paclitaxel micelles	Epithelial ovarian cancer	2015 in the Russian Federation
MM302	Merrimack Pharmaceuticals, Inc.	HER2-targeted PEGylated Doxorubicin liposomes	Advanced HER2-positive breast cancer	Phase II completed

Figures:

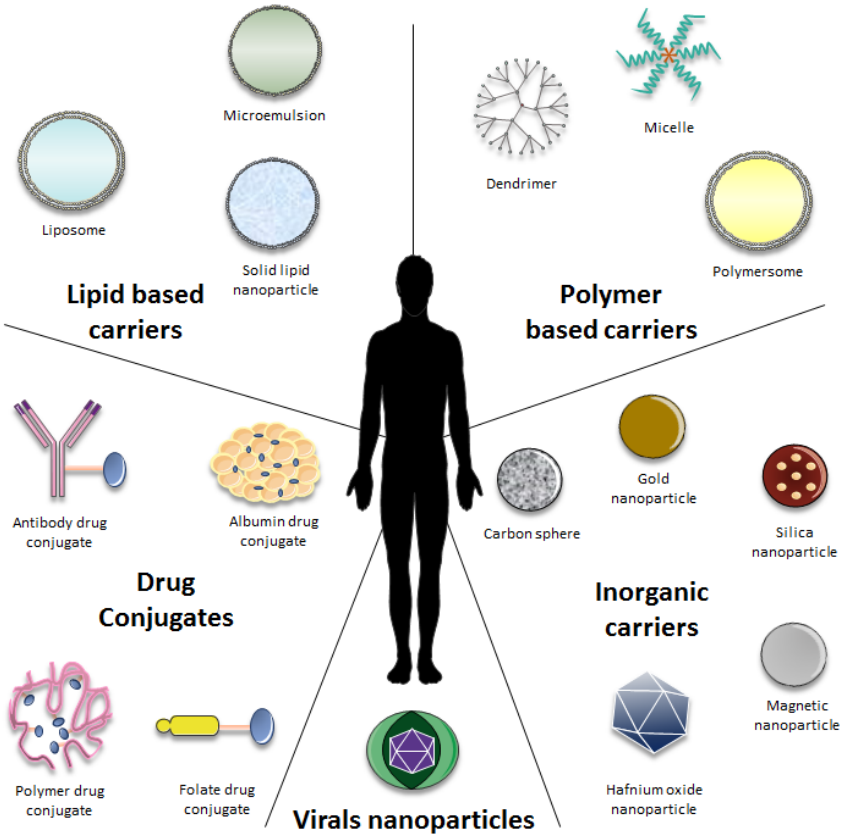

Fig.1: Classification of the main nanoparticles. Adapted from Nanomedicine in cancer therapy: Challenges, opportunities, and clinical applications**,** Journal of Controlled Release Volume 200, 28 February 2015, Pages 138–157 (Andreas Wick and al.)

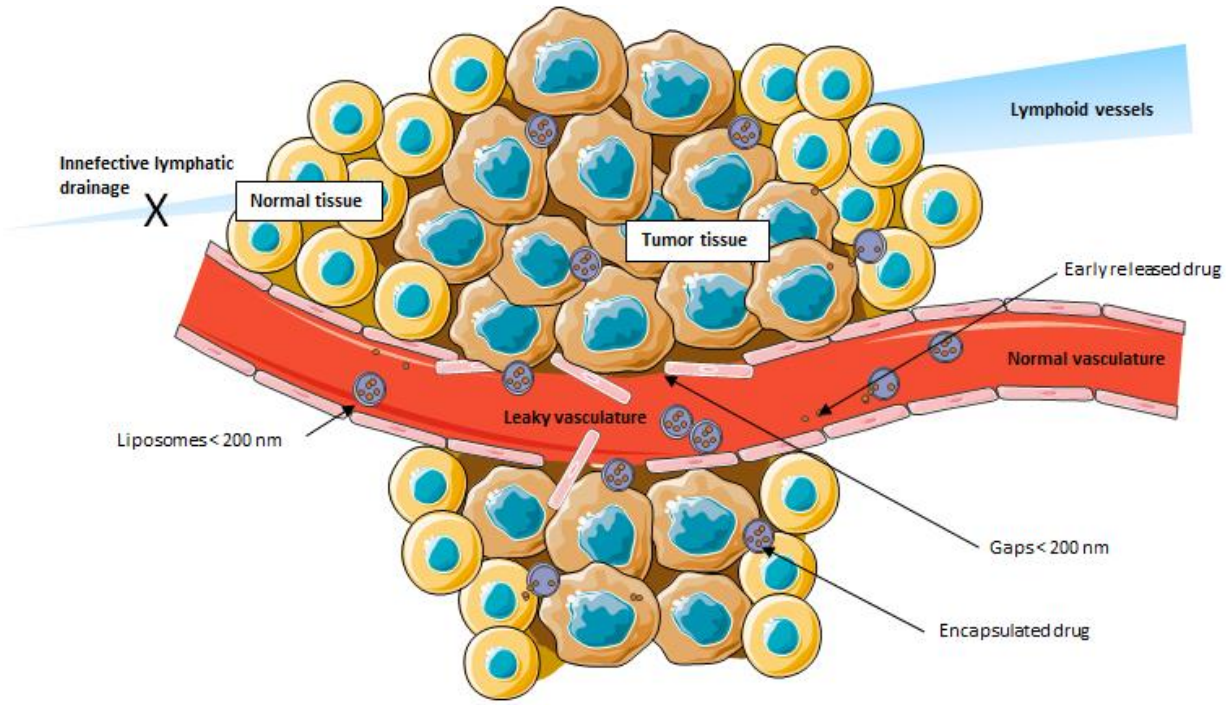

Fig.2: Schematic representation of the EPR Effect.

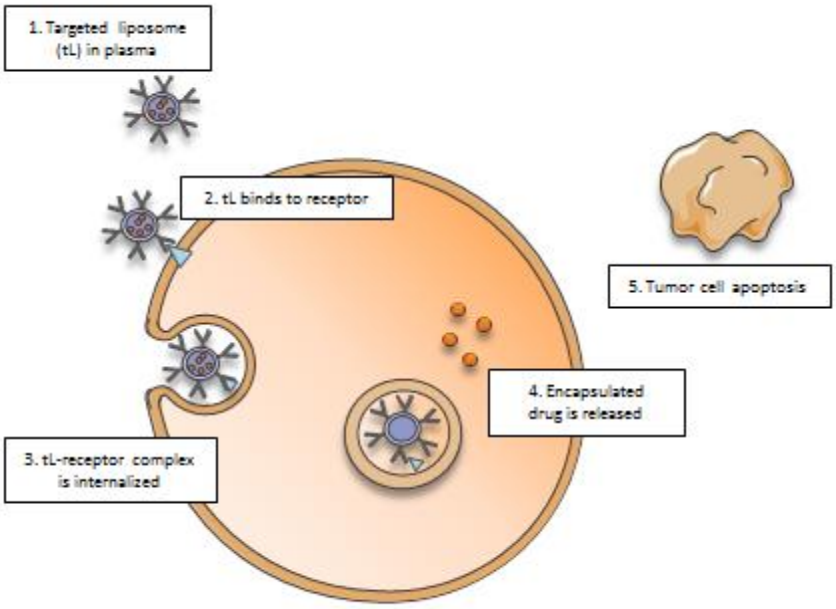

Fig.3: Schematic representation of active tumor cells targeting for immunoliposomes.

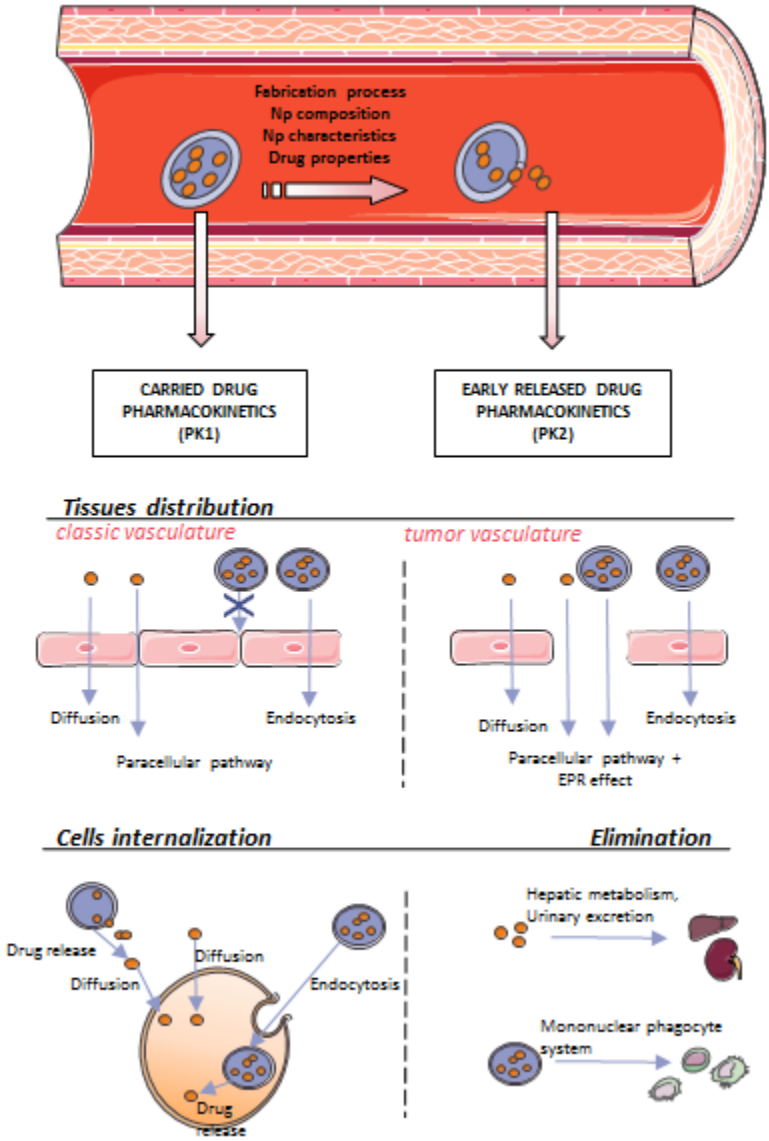

Fig.4: Schematic representation of pharmacokinetic differences between free, encapsulated and released drugs.

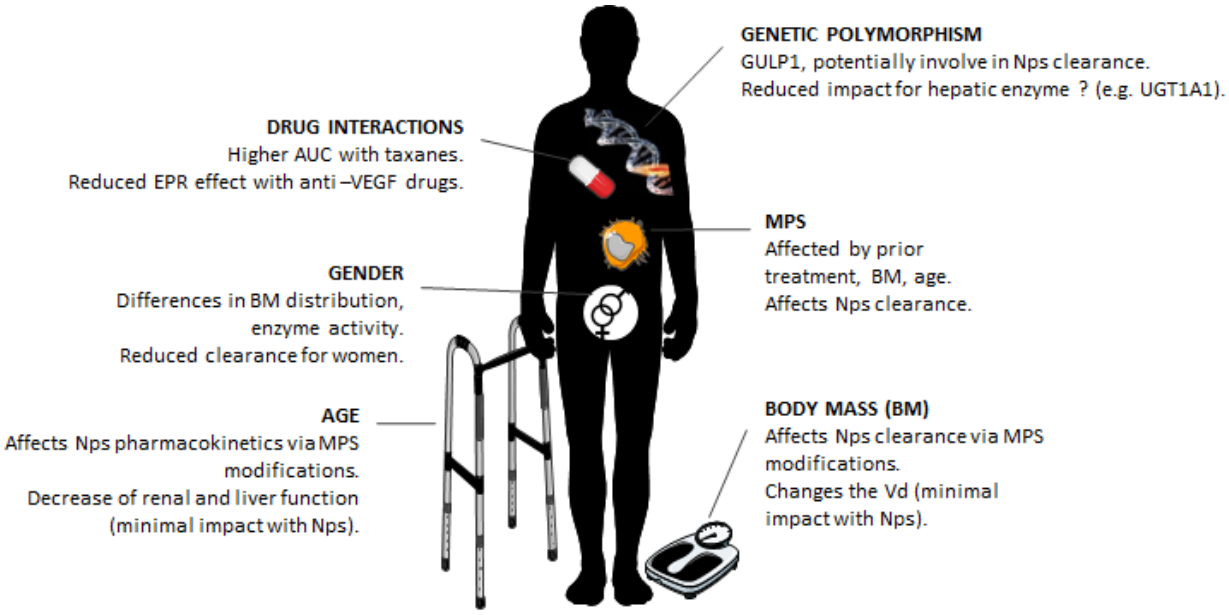

Fig.5: Schematic representation of patient factors affecting nanoparticle pharmacokinetics.

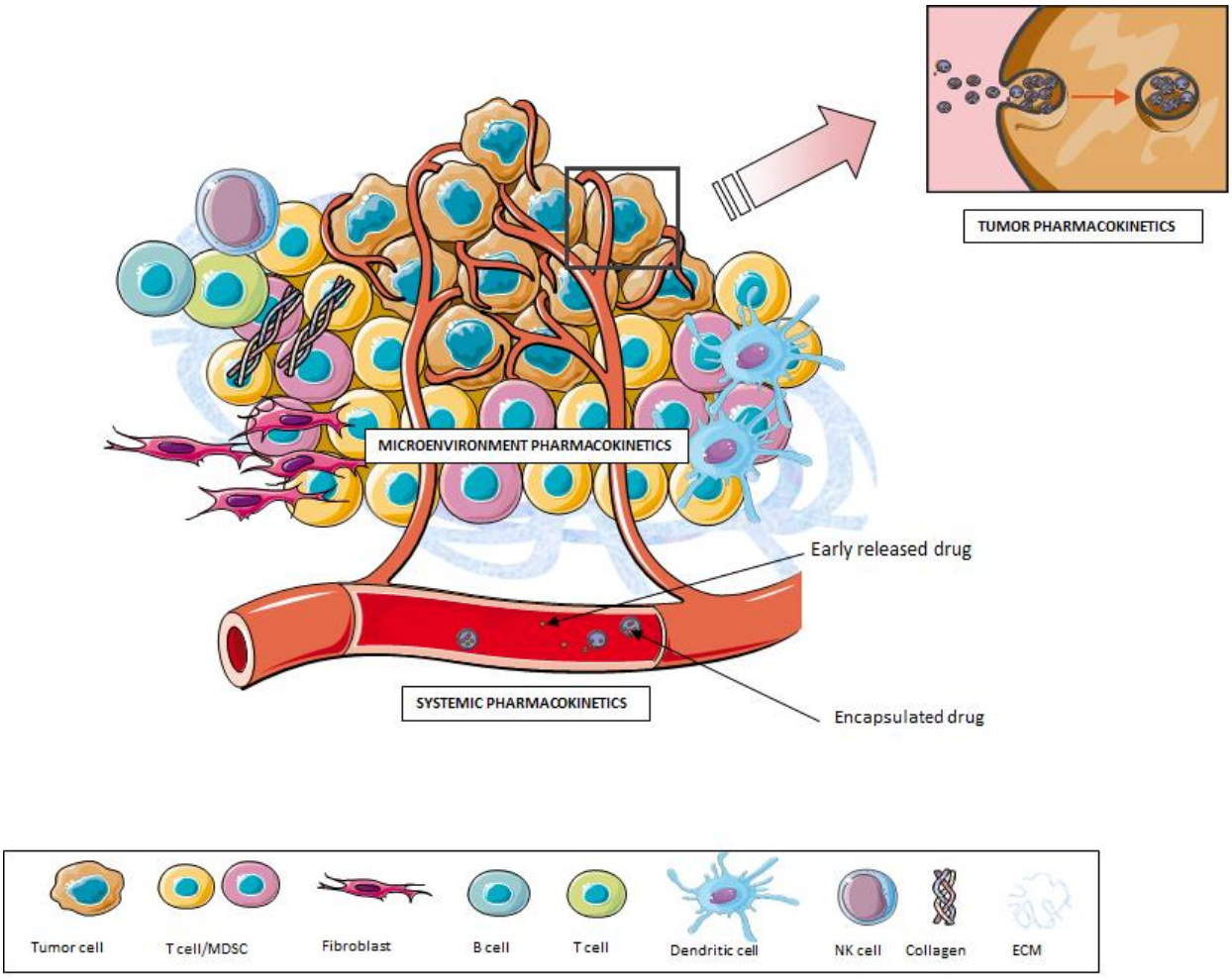

Fig.6: Schematic representation of the different pharmacokinetics to study for antitumor nanoparticles.