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Optimal physicochemical properties of antibody-nanoparticle conjugates for improved tumor targeting

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Abstract

Tumor-targeted antibody-nanoparticles (NPs) represent an innovative strategy to improve the local delivery of small molecules in comparison to untargeted NPs. However, the optimal design between the physicochemical properties of the NP (*i.e.*, size, charge, structure) and the targeting antibody moiety (*i.e.*, fragment – Fab', F(ab')₂, full monoclonal antibodies (mAb)) remains to be optimized. Here, we performed a meta-analysis of 190 peer-reviewed research articles describing the use of tumor-targeted antibody-NPs from 2009 to 2021. We first confirmed that the use of tumor-targeted antibody-NP conjugates significantly increases the tumor uptake of NPs in comparison to passively targeted NPs ($3.2 \pm 0.6\% \text{ID/g}$ vs. $7.9 \pm 1.9\% \text{ID/g}$, $p\text{-value} = 4.07 \times 10^{-10}$); second, this study demonstrates that for lipidic NP, the use of a fragment antibody provides higher tumor uptake than a full mAb conjugation strategy ($p\text{-value} < 10^{-4}$) and that for a polymer and organic/inorganic NP, the use of a full mAb provide higher tumor uptake than fragment conjugation strategy ($p\text{-value} < 10^{-3}$ and $< 10^{-4}$, respectively); third, for both lipidic and polymeric NP, the use of smaller NP leads to an improved tumor uptake of the NPs ($p\text{-value} < 10^{-4}$, respectively); finally, we demonstrated that the pharmacokinetic parameter of the conjugates is driven by the NP and not by the antibody moiety, confirming the importance to rationalize the design of the NPs for such purpose.

Keywords: antibody; biodistribution; nanomedicine; pharmacokinetics; targeted nanoparticles

Introduction

Improving the targeting specificity of small molecules has been a primary focus in cancer treatment to reduce associated toxicities and improving their therapeutic index^[1]. Antibody-drug conjugates (ADC) and nanoparticles (NPs) have been developed to fulfill these roles; while the former is widely accepted in the clinic with routine applications for the treatment of hematological malignancies and breast cancers with an effective anti-tumoral activity^[2-4], ADCs usually demonstrates a relatively low average drug to antibody ratios (DAR) – *i.e.* 2 and 4 – to prevent recognition by scavenger B receptor in the liver. At the opposite, NPs relied mostly on the use of the passive targeting route to increase the delivery of small molecules to the tumor^[5,6]. Depending on their physicochemical structures, they are able to conjugate up to 10 times more small molecules than the ADCs. However, the passive tumor targeting route was demonstrated to be limited with an average of 0.7% of the injected dose (ID)^[7] and with large heterogeneity between the tumor types but also within the same patients tumors^[8-12].

As such, it was a logical step to sought to combine the specificity of targeting of ADCs with the drug loading ability of NPs. Several strategies have been studied using full monoclonal antibodies (mAbs) conjugates (full mAb-NPs) and fragments of antibody decorating NPs (fragment-NPs).

Through this meta-analysis, we aimed to identify the optimal antibody moiety-NPs combination leading to the highest NP tumor uptake upon a rational selection based on the material of the NPs (lipidic, polymeric, and organic/inorganic); we focused particularly on the size of the NPs, the antibody targeting moieties (full mAb, fragments), the conjugation strategy, and the overall impact of these parameters on the pharmacokinetic and non-specific biodistribution of these full

mAb-/fragment-NPs. The search term ‘antibody-targeted nanoparticles AND tumor targeting’ were used to identified 190 peer-reviewed publications between 2009 and 2021, including 108 previously listed in the perspective article from Wilhelm *et al.*^[7] and 82 new peer reviewed publications assessing antibody-targeted NPs (**Figure 1**) (**Supplementary Table 1**) in the PubMed and Google Scholar databases. Among the selected peer-reviewed articles, 62/82 are using full mAb-NPs conjugation strategy to improve the NP tumor uptake, and 18/82 are using fragment-NP conjugation strategy for this role. 18/82 are lipidic-based NPs, 27/82, are polymeric NPs, and 33/82 are organic/inorganic-based NPs.

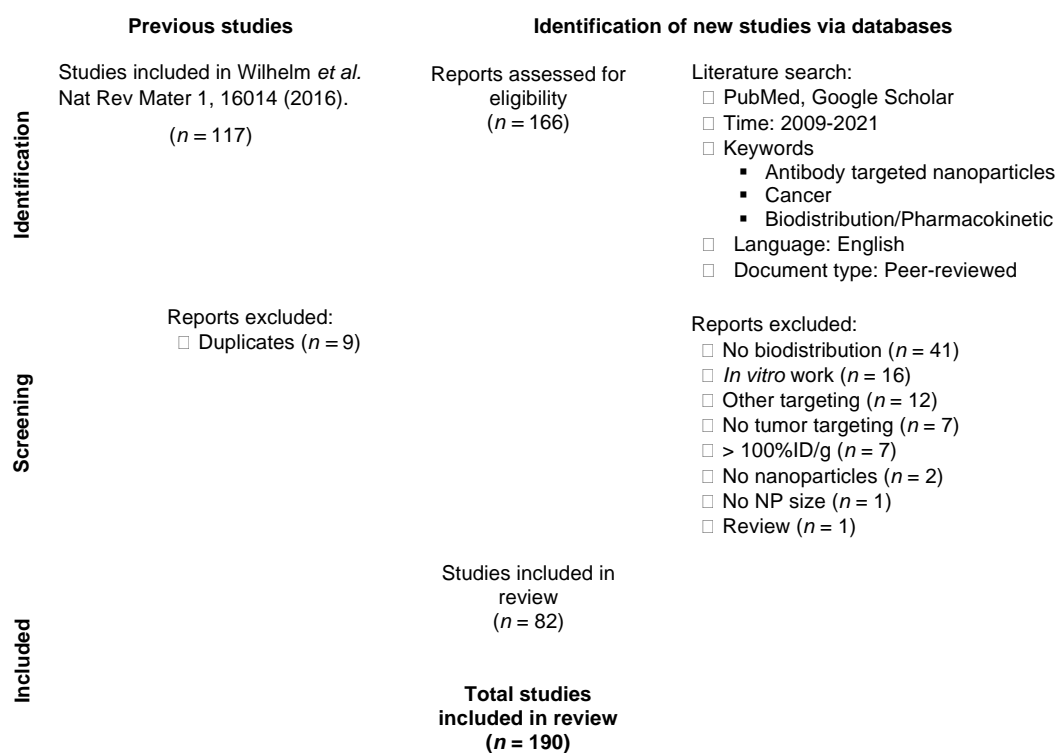


Figure 1: Procedure, strategies, and inclusion criteria for the literature search. Following the literature search from the databases of PubMed and Google Scholar, 190 peer-reviewed publications between 2009 and 2021 were identified for subsequent BD/PK meta-analysis.

1. Nanoparticles dictate the pharmacokinetics and biodistribution profiles of antibody- /fragment-targeted NPs conjugates

Among the 82 publications presenting either full mAb-NPs or fragment-NPs, only 40% (33/82) presented a pharmacokinetic (PK) study (**Supplementary Table 2**), 22% (18/82) provided enough information to calculate the $AUC_{0-\infty}$ of the conjugate, 11% (9/82) for the C_{max} , and 40%, (33/82) presented the half-life of the NPs in the blood. No statistical differences (p -value = 0.69, 0.95, and 0.29, respectively, Mann-Whitney test) were observed for any of these parameters between the non-targeted NPs and the full mAb-/fragment-targeted NPs (**Figure 2A-B, Supplementary Figure S1**).

Interestingly, we observe a higher variability of the half-life for the full mAb-NP conjugates (p -value = 0.0013, Fisher-Snedecor test) (**Figure 2B**) than for the fragment-NP conjugates. Such variabilities are observed for the -ximab isotype full mAb-NPs (p -value = 0.0029, Fisher-Snedecor test) (**Figure 2C**), but not for -omab full mAbs isotopes, nor for the fragments-NP conjugates (p -value = 0.79, and 0.74, respectively, Fisher-Snedecor test) (**Figure 2B-C**).

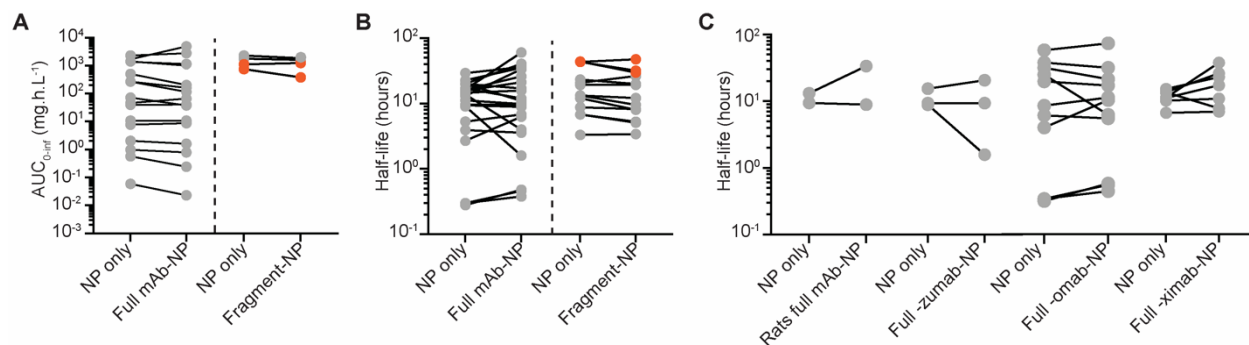


Figure 2: Analysis of the pharmacokinetics parameters of antibody moiety-targeted nanoparticles. **A.** $AUC_{0-\infty}$ before and after conjugation of the NP to a full-monoclonal antibody (mAb) or to a fragment. **B.** Half-life before and after conjugation of the NP to a full-monoclonal antibody (mAb) or to a fragment. Orange dots represents the data extracted from nanoparticles currently in clinical trials. No statistical differences were observed between the groups, Mann Withney test.

The half-life variabilities observed for the full -ximab-NPs could be attributed to either the antibody-driven PK interindividual variability^[13–15] justified by the injection of chimeric antibodies in murine model, and/or the amount of NPs per mAb which can fluctuate based on the conjugation route employed, and/or the NPs site-conjugation on the antibodies – *i.e.* unspecific conjugation of the NP onto the Fc or the Fab part of the mAb.

The lack of significant half-life PK variabilities for the -zumab-NP is mostly attributed to the size of the cohort analyze. The role of the linker used to conjugate the NPs has been evaluated as well (**Supplementary Figure S4**). We observed significant half-life variabilities for the thiol conjugation approach, but not for the amine conjugation strategy (p -value = 0.0042, and 0.16, respectively, Fisher Snedecor test, **Supplementary Figure S4**). Additionally, the impact of the number of NPs per mAb should be an additional factor to be considered, however, such information is rarely explicitly mentioned. As such, this variable was removed from this analysis.

This PK analysis confirms that the use of the targeting antibody moiety does not significantly improve the circulation time of the NPs in comparison to the non-conjugated NPs. All the half-life of these NP conjugates are orders of magnitude below the usual half-life of mAbs in the blood (~few weeks), confirming that independently on the size of the NPs, the PK of the conjugates is mostly driven by the NPs.

We then analyzed the biodistribution of the passive and antibody/fragment-NPs in the 3 major organs known to eliminate the NPs of the body – *i.e.*, liver, spleen, and kidneys (**Figure 3**). Compared to the naked NP, no clear differences are observed whether the NP is conjugated to a full mAb or to a fragment of antibody. Similarly to previously published studies deciphering the physicochemical properties of NPs and their relationships to organ accumulation, we observe a

similar biodistribution than for naked NP, where the largest complexes tend to accumulate in the liver (**Figure 3A**) and the smallest accumulate mostly in the kidneys (**Figure 3C**)^[16]. No differences were observed within the full mAb NP conjugates and fragments-NPs based on the NP material (**Supplementary Figure S3**). The charge of the antibody-/fragment-NP complex being mostly dictated by the NPs, similar biodistribution profile than for the naked NPs is observed (**Supplementary Figure S4**). Interestingly, the vast majority of these studies are using negatively charged NPs to generate the conjugation with the mAbs/fragments to reduce as much as possible the passive internalization of the conjugates in major organs and to optimize the specificity of the mAb/fragments. As a result, independently on the use of a full mAb-/fragment-NPs, the total accumulation of NPs in each of these 3 organs (liver, spleen, kidneys) is similar to the naked NPs (**Figure 3D-F**), confirming that the NP properties drive the biodistribution properties of the targeted complex.

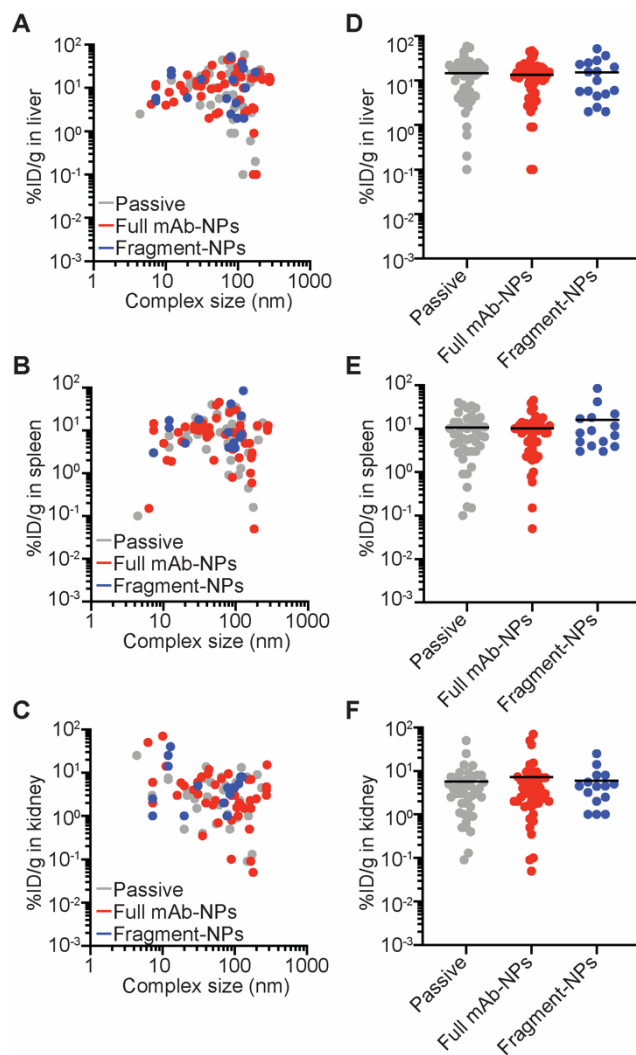


Figure 3. The physicochemical properties of the NP drive the biodistribution of the antibody-/fragment-NP complex.

2. Full antibody and fragments conjugated NPs improve tumor uptake of the NPs

It was previously demonstrated that the use of targeting NPs improve the tumor uptake in comparison to passive targeting^[7]. While no clear patterns of tumor uptake biodistribution is observed based on the size of the complex antibody moiety-nanoparticle (**Figure 4A**), the use of full mAb-NPs and fragment-NPs significantly improves the accumulation of the NP into the tumor compared to the non-targeted NPs ($7.9 \pm 1.9\%$ ID/g vs. $3.2 \pm 0.6\%$ ID/g; p -value = 4.07×10^{-7}).

¹⁰, Mann-Whitney) (**Figure 4B**). The type of targeting moieties, *i.e.*, full mAb *vs.* fragments, provide a comparable NP tumor uptake ($8,2 \pm 2,4$ %ID/g *vs.* $6,2 \pm 1,8$ %ID/g) (**Figure 4B**).

Despite the non-specificity of the lysine, thiol, or carboxylic acid conjugation strategies employed to generate full mAb-NP conjugates, no statistical differences in tumor uptake are observed between these different conjugation strategies (**Figure 4C**), nor between the different full mAb isotype used (**Figure 4D**).

Additionally, and importantly, the preclinical tumor model employed to evaluate these conjugates did not contribute to any statistical tumor uptake differences (**Figure 4E**) between the tumor-targeted NP conjugate groups.

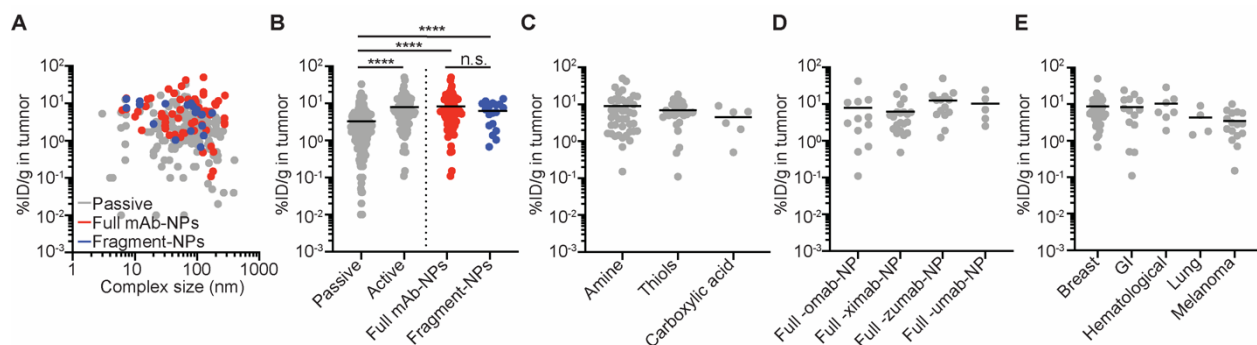


Figure 4: Analysis of antibody-targeted nanoparticles tumor uptake efficacy between 2009 and 2021. Black solid lines indicate the mean values. *P*-values are calculated with a Mann-Whitney statistical test.

Finally, we further performed an unbiased univariate analysis to confirm these results (**Figure 5, Supplementary Figure S5-6**). The analysis confirmed that the use of a targeting approach led to a better NP tumor uptake independently on the material used. However, for both polymeric and organic/inorganic NPs, the conjugation to full mAbs provide better NP tumor uptake than fragments-NPs, with no real impact of size of the complex. At the opposite, for the lipidic NPs, fragments-NPs provides increased tumor uptake with a direct relationship with the size of the complex.

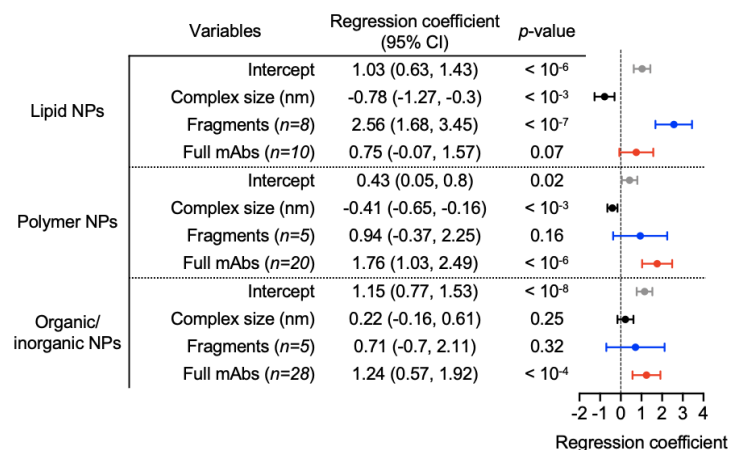


Figure 5: Unbiased univariate analysis confirms that the NP size and the type of NPs influence the tumor targeting efficiency of full mAbs/fragment-NP conjugates.

Conclusion

Anti-cancer treatments remain associated with major adverse effects. Several approaches have been developed to improve the therapeutic index and the PK profiles of chemotherapies. Antibody-targeted NPs represent an innovative strategy to increase the delivery of these molecules into tumors. Targeting NPs with antibody or fragments do improve their uptake into the tumor compared to non-targeted NPs. However, the pharmacokinetics and biodistribution profiles of antibody-targeted NPs conjugates is still dictated by the NPs. Of interest, the size of the antibody targeting moieties does not directly impact their abilities to target the tumor cells nor impact the PK properties of the NPs. This confirms the rational selection of tumor targeted fragments (scFv, F(ab')₂, etc.) rather than full mAbs for clinical development (**Table 1**).

Name	Size (nm)	Payload	Target	Clinical indications	Phases
MM-302	75-110	Doxorubicin	scFv anti-HER2	HER2 ⁺ breast cancer	Phase II ⁺
C225-ILs	85	Doxorubicin	Fab' anti-EGFR	TNBC EGFR ⁺	Phase II
MCC-465	140	Doxorubicin	F(ab') ₂ anti-GAH	Metastatic gastric cancer	Phase I [▲]
SGT-53	90	Plasmid <i>TP53</i>	scFv anti-TfR	Pediatric solid tumors	Phase I
				Pediatric CNS tumors	Phase I
				Recurrent glioblastoma	Phase II
				Metastatic pancreatic cancer	Phase II
SGT-94	90	Plasmid <i>RB94</i>	scFv anti-TfR	RB negative tumors	Phase I

Table 1. Antibody-conjugated nanoparticles in clinical trials. C225-ILs: anti-EGFR immunoliposomes; TNBC: triple negative breast cancer; TfR: transferrin receptor; CNS: central nervous system; + discontinued for clinical ineffectiveness; ▲ loss of funding.

Indeed, depending on the residues relative abundance and accessibility (100 lysyl and 34 cysteYL residues in a human IgG^[17-19]), controlling the conjugation ratio might appear challenging thus leading to the production of multiple forms of antibodies-targeted NPs with distinct BD/PK properties in every batch^[20]. Also, according to the material, the size of the NPs might also impact their tumor uptake. Further studies have to be performed to unravel this effect. As a results, to date, only a few tumors targeted NP conjugates are being evaluated in clinical trials (**Table 1**); all of them are based on the use of fragment-lipidic NPs as such conjugation allow to avoid the site-specific conjugation method issue that can be encounter between the synthesis batches (*vide supra*).

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