Design of smart peptide-based nanoparticles for siRNA delivery in gastrointestinal stromal tumors (GIST)

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1. Gastrointestinal Stromal Tumor (GIST)

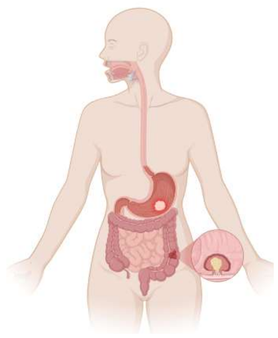
FIGURE 1

Epidemiology
- ➤ **10-15** per million/year (FR) (1)
- ➤ Most common gastrointestinal sarcomas

Molecular Basis
- ➤ Primary KIT or PDGFRA mutations in **85%** (2)

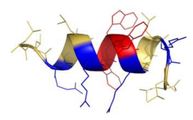
First-Line Treatment
- ➤ Imatinib (IM)

Resistance Mechanism
- ➤ **~90%** develop IM resistance
- ➤ Due to secondary KIT mutations



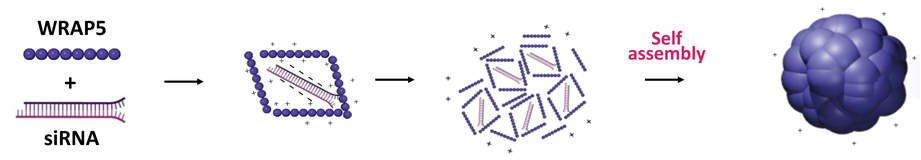
2. WRAP-based nanoparticles for delivery

FIGURE 2



WRAP5 (W5):
- 15 amino acids
- Rich in : **Tryptophan (W)** and **Arginine (R)**
- Amphipathic peptide

ID	Sequences	AA	Charges	Leucine (L)	Tryptophan (W)	Arginine (R)
W5	LLRLLRWWWRLLRLL	15	5	8	3	4



- Stable nanoparticles
- ~60-150 nm
- Fast cellular internalization
- Direct translocation

3. pH-sensitive PEG-WRAP5 nanoparticles

FIGURE 3

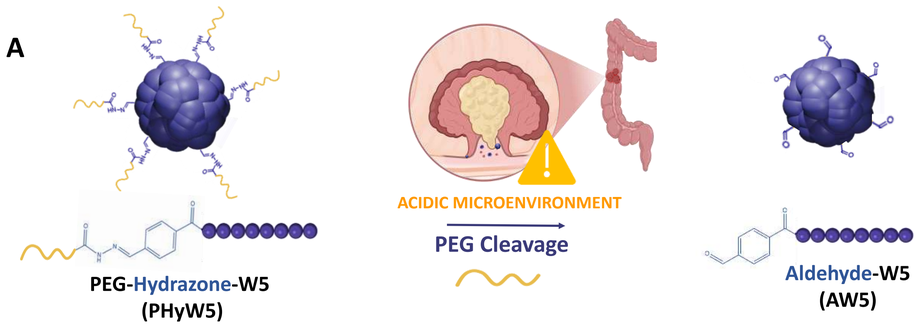
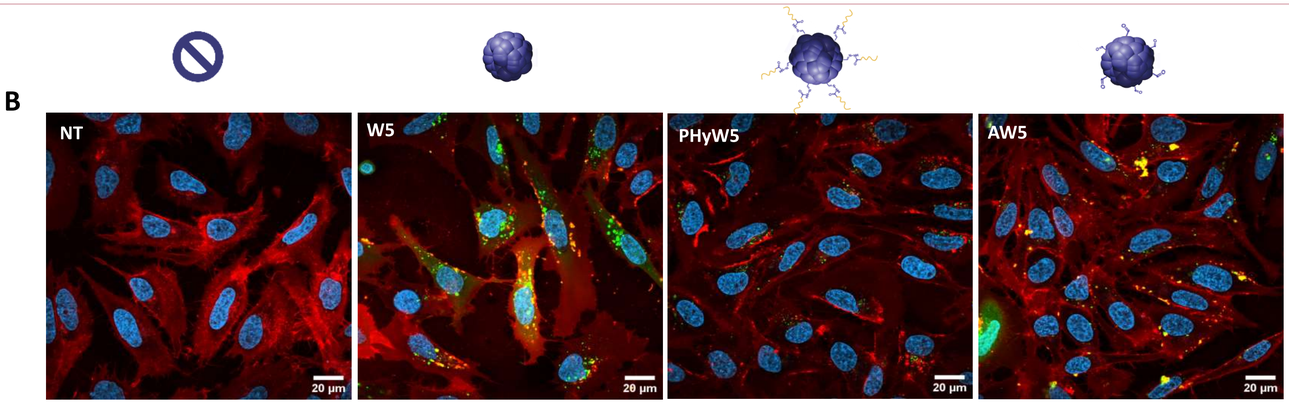


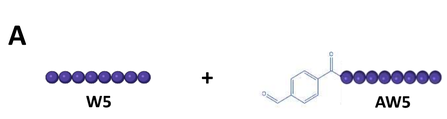
Figure 3. The use of pH-sensitive PHyW5 nanoparticles to target GIST.
(A) Principle of the pH-dependent cleavage reaction of PEG-Hydrazone-W5 (PHyW5) in the acidic environment of a GIST tumor. Hydrazone linker cleavage generates a W5 peptide carrying a 4-carboxybenzaldehyde group at its N-terminus, referred to as aldehyde-W5 (AW5).



(B) Visualization of **siCDK4-Alexa488 (green)** internalization in GIST-T1 cells after 2 h transfection with W5, PHyW5, or AW5 nanoparticles, compared to NT cells (Live Cell Confocal Microscopy). **Nuclei stained with Hoechst (cyan),** membranes with **DeepRed CellMask (red).** Efficient cytoplasmic internalization of siCDK4 using W5 nanoparticles, nearly no detectable siCDK4 internalization with PHyW5 nanoparticles, and membrane colocalization of siCDK4 with the AW5 nanoparticles.

4. Impact of AW5 on nanoparticle activity in GIST cells

FIGURE 4



PBN:siCDK4 (40 nM)			Size [d.nm]	PdI
W5	PHyW5	AW5		
100%	0%	0%	70.6 ± 9.9	0.218 ± 0.035
0%	0%	100%	90.9 ± 24.7	0.258 ± 0.191
25%	0%	75%	84.9 ± 42.8	0.239 ± 0.048
50%	0%	50%	61.2 ± 3.1	0.207 ± 0.036
80%	0%	20%	79.5 ± 9.8	0.192 ± 0.026
95%	0%	5%	85.1 ± nd	0.253 ± nd
0%	100%	0%	66.0 ± 7.9	0.324 ± 0.052

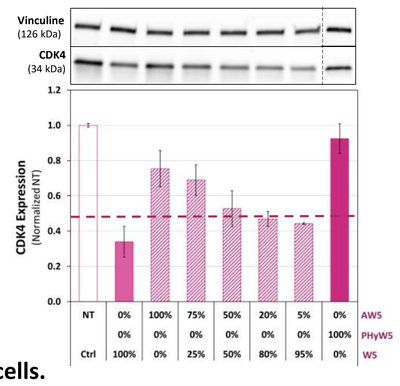
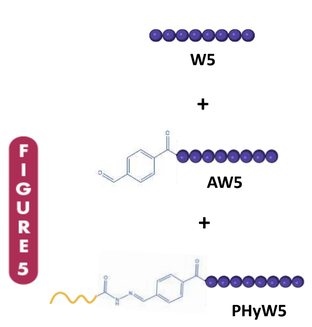


Figure 4. Evaluation of the AW5 nanoparticle activity in GIST cells.
(A) DLS characterization of siCDK4-loaded nanoparticles with variation in W5/AW5 ratios. Measurements revealed sizes of ~65 – 91 nm and PdI values of ~0.25. **(B)** Quantification of CDK4 protein levels in GIST-T1 cells transfected for 48 h with siCDK4-loaded nanoparticles with variation in W5/AW5 ratios. Data are expressed as mean ± SD. (n = 2-3). CDK4 silencing ≥50% was observed only with nanoparticles composed of less than 70% AW5.

5. Optimizing [W5/PHyW5/AW5] ratio for protection and internalization

FIGURE 5



PBN:siCDK4 (40 nM)			Size [d.nm]	PdI
W5	PHyW5	AW5		
100%	0%	0%	70.6 ± 9.9	0.218 ± 0.035
0%	0%	100%	90.9 ± 24.7	0.258 ± 0.191
0%	100%	0%	66.0 ± 7.9	0.324 ± 0.052
30%	70%	0%	193.9 ± nd	0.288 ± nd
	50%	20%	70.6 ± 2.4	0.189 ± 0.025
	40%	30%	92.2 ± 20.7	0.196 ± 0.003
	30%	40%	72.9 ± 1.3	0.184 ± 0.021
	20%	50%	63.3 ± 12.7	0.215 ± 0.023
	10%	60%	62.9 ± 2.4	0.237 ± 0.019
	0%	75%	84.9 ± 42.8	0.239 ± 0.048
50%	50%	0%	63.5 ± 3.8	0.221 ± 0.032
	40%	10%	62.4 ± 6.9	0.206 ± 0.021
	30%	20%	66.3 ± 3.5	0.185 ± 0.023
	20%	30%	65.3 ± 8.9	0.220 ± 0.02
	10%	40%	87.4 ± 34.6	0.231 ± 0.006
	0%	50%	61.2 ± 3.1	0.207 ± 0.036

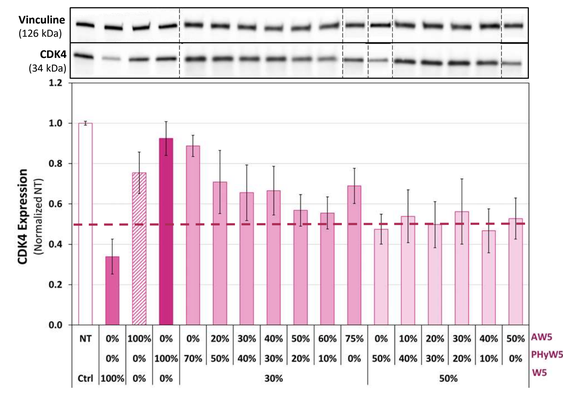


Figure 5. Evaluation of the three-peptide co-formulation activity in GIST cells.
(A) DLS characterization of co-formulated PBNs with variation in [W5/PHyW5/AW5] ratios and encapsulated siCDK4. Measurements revealed sizes of ~60- 193 nm and PdI values of ~0.20. **(B)** Quantification of CDK4 protein levels in GIST-T1 cells transfected for 48 h with the co-formulated nanoparticles. Data are expressed as mean ± SD. (n = 2-3). A knockdown of ~50% was observed for all nanoparticles containing 50% W5, as well as for those with ratios of [30%W5/20%PHyW5/50%AW5] or [30%W5/10%PHyW5/60%AW5].

Conclusion

WRAP5-Based Nanoparticle Optimization

- **WRAP5 nanoparticles are effective carriers for siRNA delivery in GIST.**
- **AW5 alters nanoparticle-cell interactions and limits silencing when used alone.**
- **Maintaining ≥30% W5 restores efficient intracellular delivery.**

➤ **The W5/PHyW5/AW5 strategy combines pH responsiveness with preserved biological activity.**

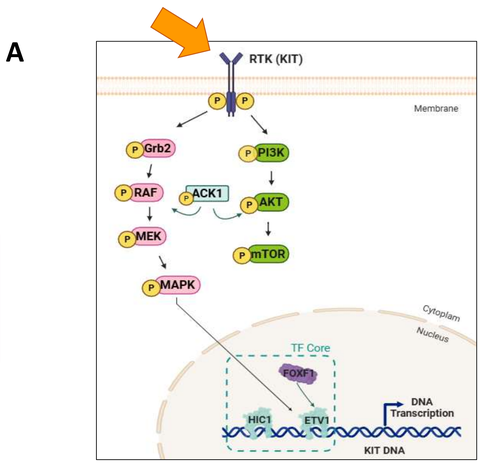
Choice of the Target and siRNA Design

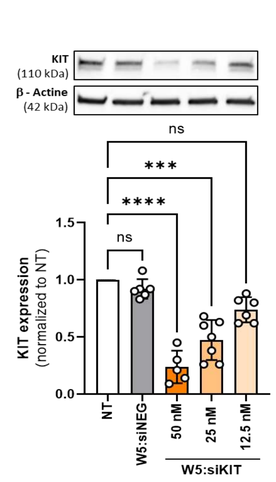
- **W5 nanoparticles efficiently deliver siRNA and achieve ~75% KIT silencing in GIST cells.**
- **More than 80% of proliferation inhibition with W5:siKIT**

➤ **Potent therapeutic tools.**

6. Targeting KIT-dependent proliferation pathways in GIST cells

FIGURE 6





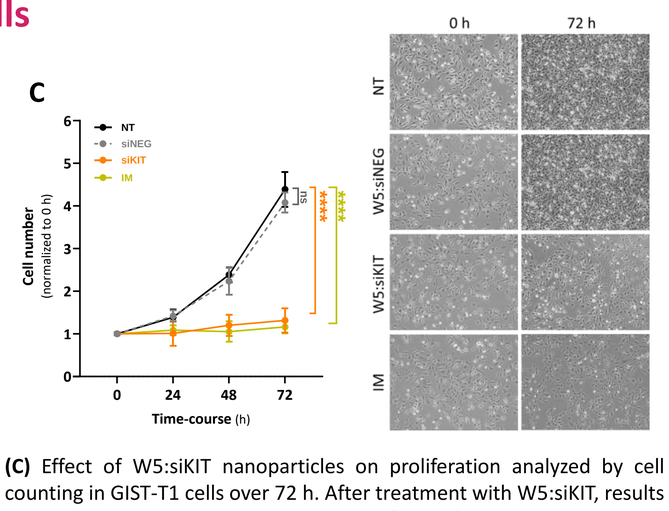
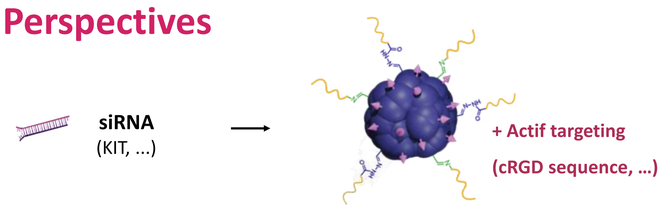


Figure 6. Impact of KIT Receptor Silencing on Cell Proliferation in GIST .
(A) Schematic overview of KIT signaling in GIST, showing MAPK and PI3K/AKT/mTOR pathway activation and regulation of core transcription factors: ETV1, FOXF1, HIC1. **(B)** Quantification of KIT protein levels in GIST-T1 cells transfected with increasing concentrations of W5-based nanoparticles encapsulating siKIT. Results were compared with untreated cells (NT) and W5:siNEG-treated cells. Data are expressed as mean ± SD. (n = 5-6) After 24 h of transfection, W5:siKIT showed ~75% protein silencing with an siRNA concentration of 50 nM.

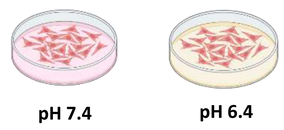
(C) Effect of W5:siKIT nanoparticles on proliferation analyzed by cell counting in GIST-T1 cells over 72 h. After treatment with W5:siKIT, results were normalized to the initial cell density (t = 0 h), then, compared with untreated cells (NT), 50 nM imatinib-treated cells (IM), and W5:siNEG-treated cells. The W5:siKIT conditions induced a significant inhibition of cell proliferation, comparable to that achieved with imatinib (IM).
Data are expressed as mean ± SD (n = 4). Statistical significance was evaluated by ANOVA (ns: not significant; ****: p < 0.0001 compared to the untreated group).

7. Perspectives

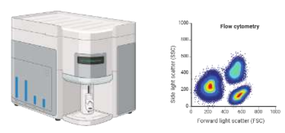


***In vitro* studies**

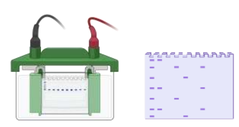
- Incubation condition



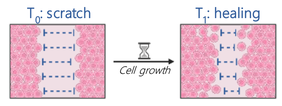
- Apoptosis induction



- Protein-silencing efficiency



- Inhibition of cell migration



References

(1) Landi B et al., Dig Liver Dis. 2019;51(9):1223-1231.

(2) Schaefer IM et al., Am Soc Clin Oncol Educ Book. 2022;42:1-15.