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TITLE: Overcoming Drug Resistance to Treat High-Risk Neuroblastomas

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CONTRACTING ORGANIZATION: Children's Hospital of Philadelphia

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14. ABSTRACT

The ultimate goal of this project is to develop a more effective yet less toxic therapy to treat children with high-risk neuroblastoma, and potentially other patients with high-risk solid tumors. We have developed a novel multivalent macromolecule, PEG-[SN22]4, that can deliver 50 times more drug to the tumor than an equivalent dose of the conventional drug, irinotecan. We can cure most or all mice in NB in mouse models with 4 weekly doses, when irinotecan cures none. Efficacy of PEG-[SN22]4 used alone or in combination will be compared to comparable doses of free or liposomal CPT-11. PEG-[SN22]4 should not only be more effective than equivalent conventional agents, but also it has been designed to produce less systemic toxicity. It is long-circulating, so exposure to normal tissues and organs is reduced, and it has been chemically modified to avoid the most common dose-limiting toxicity experienced by patients, intractable diarrhea. However, we need to demonstrate this in animal models. We also plan to investigate other agents with which PEG-[SN22]4 may be additive or synergistic, without significantly increasing toxicity. To date, we have shown considerable efficacy, curing most or all mice in transgenic, orthotopic, and PDX models, with excellent drug delivery and limited side effects.

15. SUBJECT TERMS
Neuroblastoma, drug resistance, multivalent macromolecule, nanoparticle, irinotecan, topoisomerase 1 inhibitor.

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Neuroblastoma (NB) is the most common and deadly solid tumor of childhood. High-risk NBs are characterized by intrinsic or acquired drug resistance, which is the major cause of treatment failure. We have developed a novel agent that can overcome both intrinsic drug resistance, driven by ABC transporter efflux, and acquired resistance, driven by TP53 inactivation or other mechanisms. Our multivalent macromolecule, PEG-[SN22]4, can deliver 50 times more drug to the tumor than an equivalent dose of the conventional drug, irinotecan. We plan to test this agent in clinically relevant mouse models of high-risk neuroblastoma, either alone or combined with other therapeutic agents.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Neuroblastoma, drug resistance, multivalent macromolecule, nanoparticle, irinotecan, topoisomerase 1 inhibitor,

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The ultimate goal of this project is to develop a more effective yet less toxic therapy to treat children with high-risk neuroblastoma, and potentially other patients with high-risk solid tumors. We have developed a novel multivalent macromolecule, PEG-[SN22]4, that can deliver 50 times more drug to the tumor than an equivalent dose of the conventional drug, irinotecan. We can cure most or all mice in NB in mouse models with 4 weekly doses, when irinotecan cures none. To evaluate this agent more thoroughly, we have the following specific aims:

Aim 1. We will demonstrate the efficacy and toxicity of PEG-$[SN22]_4$ to control NB growth in vivo in clinically relevant animal models of high-risk NB.

Aim 2. We will demonstrate the efficacy and toxicity of PEG-$[SN22]_4$ ±TMZ or olaparib to control NB growth in animal models of high-risk NB. Efficacy of PEG-$[SN22]_4$ used alone or in combination will be compared to comparable doses of free or liposomal CPT-11.

PEG-[SN22]4 should not only be more effective than equivalent conventional agents, but also it has been designed to produce less systemic toxicity. It is long-circulating, so exposure to normal tissues and organs is reduced, and it has been chemically modified to avoid the most common dose-limiting toxicity experienced by patients, intractable diarrhea. However, we need to demonstrate this in animal models. We also plan to investigate other agents with which PEG-[SN22]4 may be additive or synergistic, without significantly increasing toxicity.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We have developed a novel multivalent macromolecule, PEG-[SN22]4, that can deliver 50 times more drug to the tumor than an equivalent dose of the conventional drug, irinotecan. To evaluate this agent more thoroughly, we have two specific aims, mentioned above. However, in this first year of the grant, we have focused on Aim 1 and related subaims in our SOW document:

Aim 1. We will demonstrate the efficacy and toxicity of PEG-[SN22]4 to control NB growth in vivo in clinically relevant animal models of high-risk NB.

Major Task 1. We will test the efficacy of PEG-$[SN22]_4$ in heterozygous and homozygous *TH-MYCN* mouse models of NB

- We have obtained approval for mouse studies from ACURO at the DOD
- We have prepared a sufficient amount of the PEG-[SN22]4 and procured a sufficient amount of commercially available free CPT-11 (Camptosar) and liposomal CPT-11 (Onivyde) for a mouse model trial in *TH-MYCN* transgenic mice. We also tested two different doses and schedules of free CPT-11 to provide a therapeutic option that might be comparable to the liposomal CPT-11 option.
- We have carried out a 4-week trial of the above agents to treat spontaneously occurring neuroblastomas in TH-MYCN mice, initiating treatment when tumors in the abdomen were palpable.
- We completed the above therapeutic trial and followed these mice for signs of treatment toxicity or tumor progression.
- We performed necropsies on mice after six months from the initiation of therapy, and there was no evidence of recurrent or metastatic disease, even after histological examination of tissues.

This study showed the clear superiority of PEG-[SN22]4 over other therapeutic arms. It also showed that treatment with free CPT-11 at twice the dose and twice a week (instead of once) gave a result that was comparable if not better than liposomal CPT-11. Furthermore, we learned that the liposomal formulation of CPT-11 (Onivyde) was showing disappointing results in adult clinical trials, and it was becoming very difficult and expensive to obtain. Therefore, we decided to discontinue testing this arm for future studies (see below).

We wanted to assess the relative efficacy of PEG-[SN22]4 to treat the spontaneous tumors that arose in immunocompetent transgenic mice with the *TH-MYCN* transgene. Mice that are homozygous for this transgene almost all develop spontaneous tumors in the adrenal glands or in the paraspinal sympathetic ganglia in the abdomen by 6-10 weeks. We waited until tumors were readily palpable (2-3 cm^3) and then started treatment with either Irinotecan/CPT-11 or PEG-[SN22]4. We tested two different doses and schedules of free CPT-11: 15 mg/kg given once a week for 4 weeks, or 30 mg/kg given twice a week IV. We also gave liposomal CPT-11 at 15 mg/kg once a week IV. PEG-[SN22]4 was dosed at 10 mg/kg/dose, once a week for 4 weeks IV. Drug doses were determined by the amount of SN38 or SN22 delivered. Saline was given IV once a week for 4 weeks as a control. Mice were followed for 6 months or until they showed signs of distress from their tumors (losing weight, hunching, decreased activity, etc.).

The lower dose of CPT-11 did essentially nothing compared to the control group (**Figure 1**). The higher dose of CPT-11 and the liposomal CPT-11 had a similar impact on prolonging survival. All except one of the mice treated with the higher dose of free CPT-11 had to be sacrificed. However, all mice treated with PEG-[SN22]4 became nonpalpable, and they remained so for at least 6 months (5 months after the 4-week treatment period). We performed necropsies on the surviving animals, and none had gross or microscopic evidence of residual tumor. Thus, all the mice treated with PEG-[SN22]4 were cured, whereas only one mouse treated with the more intensive dose and schedule of free CPT-11 was cured.

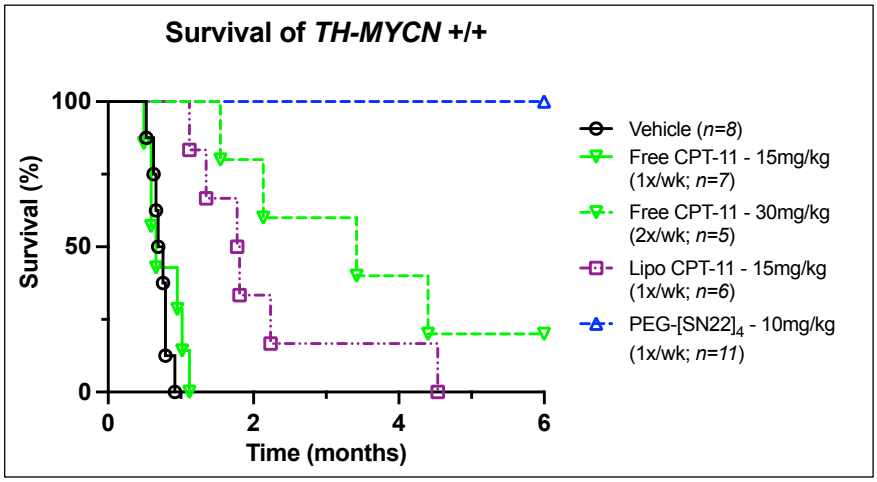

Figure 1. Efficacy of PEG-[SN22]$_4$ in a *TH-MYCN* transgenic mouse model. Homozygous *TH-MYCN* mice with palpable tumors were treated with vehicle (saline) (*n=8*), Free CPT-11 (15 mg/kg/dose once a week; *n=7*), free CPT-11 (30 mg/kg/dose twice a week, n=5)Liposomal CPT-11 (15 mg/kg/dose once a week; *n=6*), or PEG-[SN22]4 (10 mg/kg/dose once a week; *n=6*). All mice were treated IV by tail vein x 4 weeks. Treatments started when the mice were about 5 weeks old with a tumor volume about 1-2 cm^3. Mice were removed from the study when they showed signs of distress due to tumor burden. PEG-[SN22]4 had rapid tumor regression, and no tumor was found at necropsy at 180-200 days. Log-rank (Mantel-Cox) Test: SN22 vs. Vehicle: p=0.0003; SN22 vs. Free CPT-11: p=0.0004; SN22 vs. Liposomal CPT-11: p=0.0005.

Major Task 2. We will test the efficacy of PEG-$[SN22]_4$ in orthotopic xenografts of 4 NB cell lines.

We chose four NB cell lines to test in an orthotopic model of NB to represent subsets of high-risk NB, chemo-naïve and chemo resistant tumors, with and without *MYCN* amplification. We initially chose SK-N-BE(2)C as a chemo-resistant NB (obtained at relapse) that had *MYCN* amplification and mutated/inactivated TP53. Because of the difficulty following tumor growth, SK-N-BE(2)C cells were transfected with luciferase to follow tumor growth and response to treatment. Because this is a notoriously difficult line to kill, we treated with comparable doses of Irinotecan/CPT-11 or PEG[SN22]4 once a week for 8 weeks. All the mice treated with free Irinotecan/CPT-11 progressed during treatment, and there was essentially no effect on tumor growth or survival (**Figure 2**). In contrast, essentially complete regression of tumors was seen in all mice treated with PEG[SN22]4, and the tumors remained undetectable for at least 12 weeks, after which tumors began to recur. The results show a dramatic survival benefit of treatment with PEG[SN22]4, but the dose, schedule, and duration of treatment were not sufficient to cure these mice of their tumors.

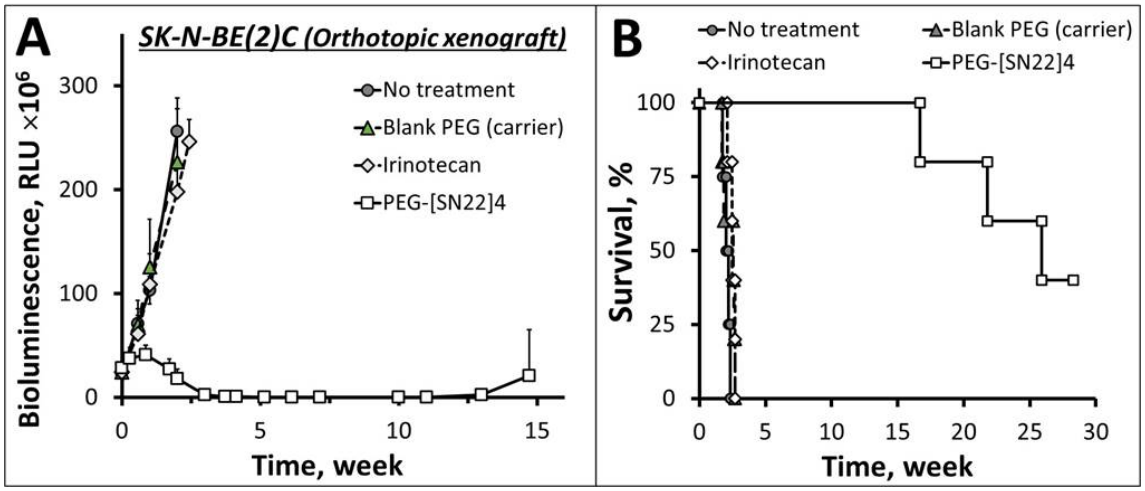

Figure 2. Efficacy of PEG-$[SN22]_4$ to treat SK-N-BE(2)C NB cells in an orthotopic mouse model. Luciferase-transfected SK-N-BE(2)C cells were implanted orthotopically in the suprarenal fat pad and followed for tumor growth by bioluminescence. Treatment was started once tumors reached 10 million RLUs. Mice were treated with either nothing, blank PEG/carrier, free Irinotecan/CPT-11 (15 mg/kg/dose once a week), or PEG-[SN22] (10 mg/kg/dose once a week) or PEG-$[SN22]_4$ (10 mg/kg/dose once a week; $n=6$). All mice were treated IV by tail vein x 4 weeks. Treatments started when the mice were about 5 weeks old with a tumor volume about 1-2 cm^3. Mice were removed from the study when they showed signs of distress due to tumor burden.

Major Task 3. We will test the efficacy of PEG-[SN22]$_4$ in 4 NB PDXs chosen to represent the two major high-risk subsets of NB.

We initially selected four different NB PDX's to represent two different molecular genetic subsets of high-risk NB: one with *MYCN* amplification and intrinsic or acquired drug resistance, and the other without *MYCN* amplification but with *ATRX* mutation. The latter subset was representative of the more indolent disease that occurs in older patients. Not all PDX lines initially selected were available initially or grew readily in our mouse model. Also, our SOW was overly ambitious in terms of the number of lines and PDX's we would be able to study in the first 12 mo, and this was further compromised by a resurgence of COVID-19 in late 2021-early 2022 which limited our ability to conduct these experiments.

We started with COG-415x, a PDX line derived from a recurrent tumor with *MYCN* amplification and an activating *ALK* mutation. Tumors were grown as flank xenografts, because we could not transfect PDX lines with luciferase for bioluminescence monitoring. Once tumors were established at 0.2 cm3 minimal size, mice were treated either with vehicle/saline, Free CPT-11 (15 mg/kg/dose), Liposomal CPT-11 (15 mg/kg/dose) or PEG-[SN22]4 (10 mg/kg/dose) all once a week IV for 4 weeks. Both free and liposomal CPT-11 prolonged survival compared to the saline control, but all mice so treated developed recurrent tumors soon after the cessation of therapy, and all mice became symptomatic and had to be sacrificed. However, all tumors disappeared in the group treated with PEG-[SN22]4, and there were no recurrences even at 6 months after the initiation of treatment. These results show the superiority of PEG-[SN22]4 to treat NBs using a PDX model, which some consider to be more clinically relevant than established NB cell lines. PEG-[SN22]4 was effective even for this NB with *MYCN* amplification, *ALK* activation, and acquired drug resistance.

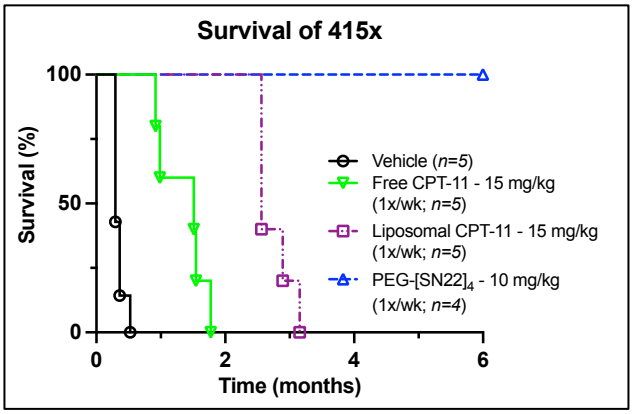

Figure 3. Treatment of PDX, COG-N-415x. Nude mice with tumors were treated for 4 weeks with vehicle (saline) (*n=8*), Free CPT-11 (15 mg/kg/dose; *n=7*), Liposomal CPT-11 (15 mg/kg/dose; *n=6*), PEG-[SN38]4 (10 mg/kg/dose; *n=6*) or PEG-[SN22]4 (10 mg/kg/dose; *n=6*) IV by tail vein 1x/week x 4 weeks.. Mice treated with PEG-[SN22]4 were tumor-free for over 6 months.

Major Task 4. We will assess toxicity of PEG-[SN22]$_4$ in multiple organs (liver, spleen, kidney lung, brain, GI tract and marrow).

- We treated 129SvJ mice with comparable doses of PEG-[SN22]4, free CPT-11 for 4-wk
- We harvested liver, spleen, kidney, lung, GI tract and brain for toxicity analysis using tissue histology after end of 4 weeks
- We obtained blood for complete blood counts and liver function tests
- We analyzed tissues for drug levels of SN22 or SN38

All mice are WT 129SvJ mice that were 6-8 weeks old at the start of treatment. The control group was treated with saline, once per week for 4 weeks. The free CPT-11 group was treated with CPT11 at 30 mg/kg twice per week for 4 weeks. The PEG-[SN22]4 group was treated with PEG-SN22 at 10 mg/kg once per week for 4 weeks. CBC and serum for liver enzymes were obtained pre-treatment, after 2 weeks of treatment, after 4 weeks of treatment, and after 6 weeks of treatment. All parameters returned to the level of control group after 6 weeks of treatment in the CPT11 and PEG-SN22 groups (see graphs below). Although tissues from treated mice have been obtained for analysis, this histological analysis for organ toxicity has not been carried out yet. Nevertheless, we have completed the analysis of blood counts and liver enzymes, as well as analyzed the drug levels in different tissues. There was transient decrease in neutrophils at weeks 2 and 4 of the study that recovered by week 6, but no evidence of liver toxicity.

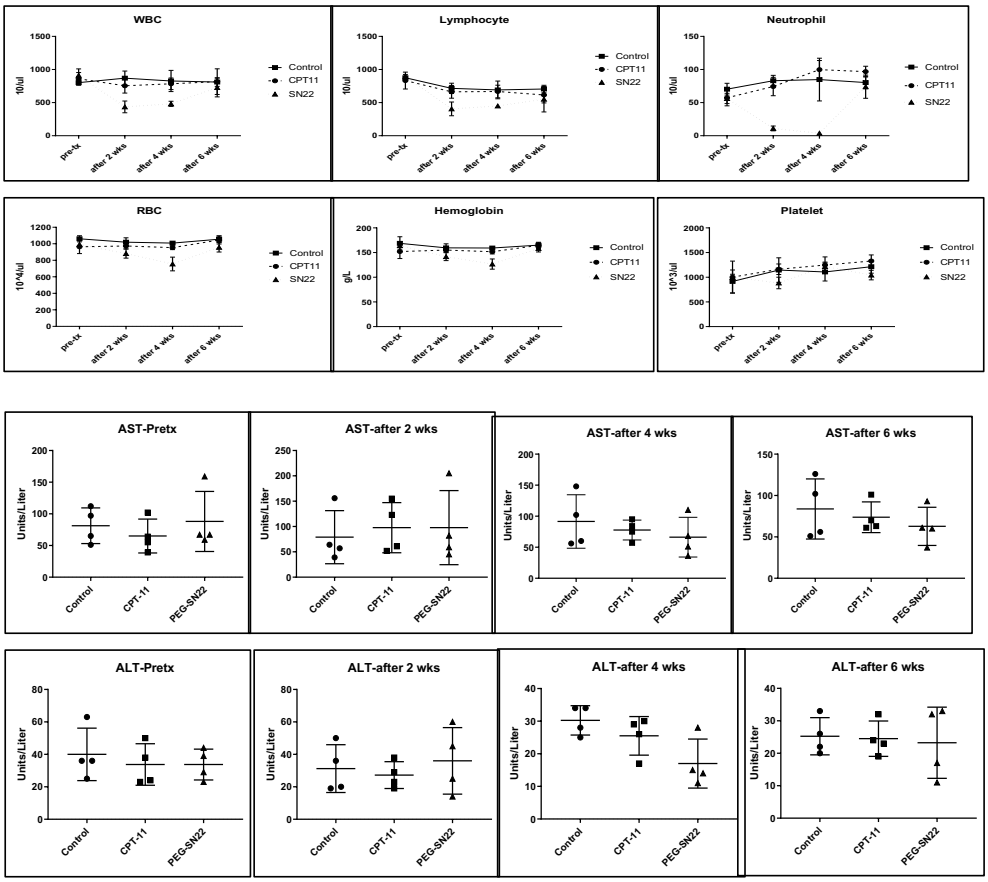

Figure 4. Toxicity to bone marrow and liver. Mice were given saline (2x/wk), free CPT-11 (30 mg/kg;2x/wk), or PEG-[SN22]$_4$ (10 mg/kg;1x/wk) for 4 weeks. Complete blood counts (CBC) performed by the Translational Core laboratory of the CHOP Research Institute.

BLOOD	Drug concentration (µg/g)			Total drug (µg)		
	4 h	24 h	72 h	4 h	24 h	72 h
Free CPT-11 (CPT-11)	1.22 ± 0.89	0.08 ± 0.04	< 0.01	1.82 ± 1.33	0.12 ± 0.06	< 0.01
Free CPT-11 (SN38)	0.13 ± 0.07	< 0.01	< 0.01	0.19 ± 0.11	< 0.01	< 0.01
Liposomal CPT-11 (CPT-11)	152 ± 9.3	16.1 ± 12.9	< 0.01	228 ± 14	24.1 ± 19.3	< 0.01
Liposomal CPT-11 (SN38)	2.62 ± 0.35	1.06 ± 0.58	0.01	3.93 ± 0.53	1.58 ± 0.88	< 0.01
PEG-$[SN38]_4$ (SN38)	8.98 ± 0.47	1.05 ± 0.15	0.3 ± 0.05	13.5 ± 0.70	1.58 ± 0.23	0.45 ± 0.07
PEG-$[SN22]_4$ (SN22)	29.5 ± 1.01	4.34 ± 0.29	0.53 ± 0.11	44.3 ± 1.51	6.5 ± 0.44	0.79 ± 0.16

TUMOR	Drug concentration (µg/g)				Total drug (µg)			
	4 h	24 h	72 h	168 h	4 h	24 h	72 h	168 h
Free CPT-11 (CPT-11)	22.5 ± 15	1.18 ± 0.21	< 0.01		35.99 ± 26	1.74 ± 0.72	< 0.01	
Free CPT-11 (SN38)	0.14 ± 0.03	0.01	< 0.01		0.22 ± 0.06	0.02 ± 0.01	< 0.01	
Liposomal CPT-11 (CPT-11)	38 ± 6.6	44.8 ± 7.7	6.8 ± 2.4		55.2 ± 11.9	30.7 ± 9.9	1.26 ± 0.22	
Liposomal CPT-11 (SN38)	0.72 ± 0.17	1.07 ± 0.22	0.27 ± 0.05		1.12 ± 0.46	0.78 ± 0.3	0.05 ± 0.01	
PEG-$[SN38]_4$ (SN38)	3.11 ± 0.33	2.99 ± 0.22	3.47 ± 0.46		2.93 ± 0.39	1.37 ± 0.41	1.17 ± 0.33	
PEG-$[SN22]_4$ (SN22)	6.67 ± 0.86	7.31 ± 0.52	26.48 ± 7.4	23.05 ± 5.25	5.06 ± 1.86	3.7 ± 1.32	3.14 ± 1.12	2.768 ± 0.37

LIVER	Drug concentration (µg/g)				Total drug (µg)			
	4 h	24 h	72 h	168 h	4 h	24 h	72 h	168 h
Free CPT-11 (CPT-11)	36.6 ± 24.5	0.91 ± 0.35	0.02 ± 0.01		44.7 ± 30	1.11 ± 0.43	0.02 ± 0.01	
Free CPT-11 (SN38)	0.72 ± 0.24	0.01 ± 0.01	< 0.01		0.89 ± 0.29	0.02 ± 0.01	< 0.01	
Liposomal CPT-11 (CPT-11)	49.6 ± 2.8	18.6 ± 8.8	0.59 ± 0.3		60.7 ± 3.5	22.8 ± 10.8	0.72 ± 0.37	
Liposomal CPT-11 (SN38)	2.62 ± 0.40	0.80 ± 0.33	0.02		3.2 ± 0.49	0.97 ± 0.41	0.02 ± 0.01	
PEG-$[SN38]_4$ (SN38)	2.13 ± 0.32	1.53 ± 0.17	2.48 ± 0.27		2.6 ± 0.39	1.87 ± 0.21	3.03 ± 0.33	
PEG-$[SN22]_4$ (SN22)	3.41 ± 0.23	4.22 ± 0.31	4.25 ± 0.54	3.115 ± 0.32	4.17 ± 0.28	5.16 ± 0.37	5.2 ± 0.66	3.599 ± 0.24

SPLEEN	Drug concentration (µg/g)				Total drug (µg)			
	4 h	24 h	72 h	168 h	4 h	24 h	72 h	168 h
Free CPT-11 (CPT-11)	184 ± 45	1.8 ± 0.96	0.01		34.15 ± 5.9	0.33 ± 0.19	< 0.01	
Free CPT-11 (SN38)	0.22 ± 0.05	< 0.01	< 0.01		0.04 ± 0.01	< 0.01	< 0.01	
Liposomal CPT-11 (CPT-11)	133.9 ± 16	67 ± 25.6	1.9 ± 0.8		21.8 ± 2	8.23 ± 2.25	0.19 ± 0.04	
Liposomal CPT-11 (SN38)	3.02 ± 0.72	1.13 ± 0.53	0.05 ± 0.03		0.49 ± 0.11	0.13 ± 0.04	< 0.01	
PEG-$[SN38]_4$ (SN38)	2.81 ± 0.27	2.50 ± 0.25	1.05 ± 0.15		0.34 ± 0.02	0.25 ± 0.06	0.23 ± 0.05	
PEG-$[SN22]_4$ (SN22)	4.71 ± 0.30	6.21 ± 0.81	7.66 ± 1.14	6.078 ± 0.65	0.55 ± 0.06	0.78 ± 0.12	0.48 ± 0.06	0.665 ± 0.08

INTESTINE	Drug concentration (µg/g)				Total drug (µg)			
	4 h	24 h	72 h	168 h	4 h	24 h	72 h	168 h
Free CPT-11 (CPT-11)	124 ± 83	4.21 ± 2.97	< 0.01		139 ± 93	4.72 ± 3.33	< 0.01	
Free CPT-11 (SN38)	9.71 ± 5.61	0.10 ± 0.05	< 0.01		10.9 ± 6.3	0.12 ± 0.06	< 0.01	
Liposomal CPT-11 (CPT-11)	13.2 ± 3.3	6.3 ± 2.07	0.33 ± 0.08		14.82 ± 3.68	6.34 ± 2.07	0.33 ± 0.08	
Liposomal CPT-11 (SN38)	1.26 ± 0.18	1.17 ± 0.43	0.03		1.41 ± 0.2	1.31 ± 0.49	0.03 ± 0.01	
PEG-$[SN38]_4$ (SN38)	2.31 ± 0.25	1.06 ± 0.17	0.39 ± 0.18		2.58 ± 1.18	1.18 ± 0.19	0.44 ± 0.03	
PEG-$[SN22]_4$ (SN22)	3.08 ± 0.51	3.20 ± 0.36	2.24 ± 0.45	0.881 ± 0.11	3.45 ± 0.57	3.58 ± 0.40	2.51 ± 0.5	0.987 ± 0.12

BRAIN	Drug concentration (µg/g)			Total drug (µg)		
	4 h	24 h	72 h	4 h	24 h	72 h
PEG-$[SN38]_4$ (SN38)	0.21 ± 0.10	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.04	0.01 ± 0.01	<0.01
PEG-$[SN22]_4$ (SN22)	0.33 ± 0.08	0.16 ± 0.01	0.18 ± 0.05	0.11 ± 0.03	0.06 ± 0.01	0.07 ± 0.02

Figure 5. Drug levels of SN38 and SN22 in different organs and tissues. We measured levels of SN38 and SN22 in blood, as well as tumor, liver, spleen, intestine and brain. We measured drug levels after a single injection at hours 4, 24, 72 and 168 hr (if drug was still measurable at 72 hr).

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and

Nothing to report.

the humanities.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will report our results in peer-reviewed publications and present at national and international meetings as soon as we have sufficient new data to warrant publication. We will not generate data that would be suitable for uploading to any publicly available database.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

PEG-[SN22]4 should not only be more effective than equivalent conventional agents, but also it has been designed to produce less systemic toxicity. It is long-circulating, so exposure to normal tissues and organs is reduced, and it has been chemically modified to avoid the most common dose-limiting toxicity experienced by patients, intractable diarrhea. The principal discipline of the project could be considered the treatment of neuroblastoma, pediatric solid tumors, or solid tumors in general. Our novel agent should be more effective and less toxic in all these disciplines and serve as a model for the development of other agents.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing new to report.
PEG-[SN22]4 and related compounds have been patented by the Children's Hospital of Philadelphia and licensed to PEEL Therapeutics for further development.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Cancer is the second leading cause of death in children, next to accidents, and the second leading cause of death in adults, next to cardiovascular disease. Our novel agent promises to be both more effective and less toxic than currently available anticancer therapies. This will not only have an impact on society by improving outcomes and reducing treatment-related side effects, but it can also serve as a model for the development of other agents with similar properties and advantages.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

We have decided not to continue to pursue or evaluate liposomal CPT-11 (Onivyde) as a control in these studies. Treatment with free CPT-11 at twice the dose and twice a week (instead of once) gave a result that was comparable if not better than liposomal CPT-11. We learned that the liposomal formulation of CPT-11 (Onivyde) was showing disappointing results in adult clinical trials, and it was becoming increasingly difficult and expensive to obtain. Therefore, we decided to discontinue testing liposomal CPT-11 for future studies

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We experienced a second wave of COVID infection in early 2022 that caused a delay in our ability to carry out experiments that were planned in Aim 1 of this study. Therefore, we will complete most or all of the planned experiments in the second year of this proposal. In addition, we overestimated the number of multi-arm mouse trials we could accomplish with current person power, so it will take additional time to complete all the trials proposed.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify

the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report, other than we plan to use the more intensive CPT-11 protocol, with 30 mg/kg/dose IV twice a week instead of the lower dose and schedule (15 mg/kg IV once a week), and to drop the liposomal CPT-11 arm as being no better than the more intensive free CPT-11 arm. This will reduce the number of mice we use for the proposed studies, but still allow us to accomplish our goal of fully evaluating the efficacy and safety of PEG-[SN22]4.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Nothing to report

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

- I.S. Alferiev, D.T. Guerrero, D. Soberman, P. Guan, F. Nguyen, V. Kolla, I. Fishbein, B.B. Pressly, G.M. Brodeur, M. Chorny: Nanocarrier-based delivery of SN22 as a tocopheryl oxamate prodrug achieves rapid tumor regression and extends survival in high-risk neuroblastoma models. International Journal of Molecular Sciences 23(3): Article 1752, Feb 2022.

- I.S. Alferiev, D.T. Guerrero, P. Guan, F. Nguyen, V. Kolla, D. Soberman, B.B. Pressly, I. Fishbein, G.M. Brodeur, M. Chorny: Poloxamer-linked prodrug of a topoisomerase I inhibitor SN22 shows efficacy in models of high-risk neuroblastoma with primary and acquired chemoresistance. The FASEB Journal 36(3): e22213, Mar 2022.

PEG-[SN22]4 has been licensed to PEEL Therapeutics for development as PEEL-224. This agent is just entering Phase 1 clinical trials in adults for gastrointestinal cancers and sarcomas.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Presentations at national/international meetings or invited talks:

- Cancer Research and Drug Development–October 2021, Virtual
- Memorial Sloan-Kettering Cancer Center, Grand Rounds, November 2021, Virtual
- University of Chicago, Charles Rubin Memorial Lecture, April 2022, Virtual
- Pediatric Grand Rounds, Washington University in St. Louis, October 2022

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or

rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

No change in personnel, except that Tatiana Goodlett has been replaced by George Bratinov as a Research Technician in Dr. Michael Chorny's laboratory.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Michael Chorny (PI) 07/01/22–6/30/23
CURE Childhood Cancer Foundation
Title: Macromolecular Prodrug-based Therapy for Indolent Neuroblastoma to CURE Childhood Cancer
No overlap

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner

organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*