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PHASE I STUDIES

Pharmacokinetics of mitomycin-c lipidic prodrug entrapped in liposomes and clinical correlations in metastatic colorectal cancer patients

Alberto A. Gabizon[1,2] · Esther Tahover[1] · Talia Golan[3] · Ravit Geva[4] · Ruth Perets[5] · Yasmine Amitay[6] · Hilary Shmeeda[1] · Patricia Ohana[6]

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Summary

Background Pegylated liposomal (PL) mitomycin-c lipidic prodrug MLP) may be a useful agent in patients with metastatic colorectal carcinoma (CRC). We report here on the pharmacokinetics and clinical observations in a phase 1A/B study with PL-MLP. *Methods* Plasma levels of MLP were examined in 53 CRC patients, who received PL-MLP either as single agent or in combination with capecitabine and/or bevacizumab. MLP was determined by an HPLC-UV assay, and its pharmacokinetics was analyzed by noncompartmental methods. The correlation between clinical and pharmacokinetic parameters was statistically analyzed. *Results* PL-MLP was well tolerated with a good safety profile as previously reported. Stable Disease was reported in 15/36 (42%) of efficacy-evaluable patients. Median survival of stable disease patients (14.4 months) was significantly longer than of progressive disease patients (6.5 months) and non-evaluable patients (2.3 months). MLP pharmacokinetics was stealth-like with long T½ (~1 day), slow clearance, and small volume of distribution (Vd). The addition of capecitabine and/or bevacizumab did not have any apparent effect on the pharmacokinetics of MLP and clinical outcome. High baseline neutrophil count and CEA level were correlated with faster clearance, and larger Vd. Stable disease patients had longer T½ and slower clearance than other patients. T½ and clearance were significantly correlated with survival. *Conclusions* PL-MLP treatment results in a substantial rate of disease stabilization in metastatic CRC, and prolonged survival in patients achieving stable disease. The correlation of neutrophil count and CEA level with pharmacokinetic parameters of MLP is an unexpected finding that needs further investigation. The association of long T½ of MLP with stable disease and longer survival is consistent with an improved probability of disease control resulting from enhanced tumor localization of long-circulating liposomes and underscores the relevance of personalized pharmacokinetic evaluation in the use of nanomedicines.

Keywords Colo-rectal cancer · Liposome · Mitomycin-c · Prodrug · Pharmacokinetics · Nanomedicine

Introduction

Pegylated liposomes, sometimes referred to as Stealth liposomes, have been used to modify the pharmacokinetics and biodistribution of various anticancer agents with the aim of reducing toxicity and improving selective delivery to tumors [1, 2], and have led to clinically approved formulations for various cancer indications [3, 4]. Pegylated liposomes have an extended blood circulation lifetime as compared to traditional liposomes lacking the polymer coating, allowing systemically administered liposomal drug to reach a target tissue more efficiently. Pegylated liposomes tend to accumulate in tumors and sites of inflammation due to the enhanced permeability and retention effect (EPR) [5].

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✉ Alberto A. Gabizon
agabizon@szmc.org.il; alberto.gabizon@gmail.com

[1] Shaare Zedek Medical Center, Jerusalem, Israel

[2] The Hebrew University-Faculty of Medicine, Jerusalem, Israel

[3] Sheba Medical Center at Tel-Hashomer, Tel Aviv University, Tel Aviv, Israel

[4] Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

[5] Rambam Health Care Campus, Haifa, Israel

[6] Lipomedix Pharmaceuticals Ltd., Jerusalem, Israel

Preclinical studies of a mitomycin-c lipidic prodrug (2, 3-distearoyloxy-propane-1-dithio-4′-benzyloxycarbonyl- mitomycin-c, abbreviated as MLP) delivered by pegylated liposomes (PL-MLP) demonstrated prolonged circulation time and an improved therapeutic index as compared to free mitomycin-c (MMC) in several tumor models [6–8]. PL-MLP (Promitil®[1]) is currently under clinical development for cancer chemotherapy and chemo-radiotherapy [9, 10]. The MLP molecule was designed with a long diacylglycerol moiety to allow its intercalation in the lipid bilayer as a lipid component, resulting in a highly stable association with the liposome under storage conditions (>5 years shelf stability in buffer suspension at 5 °C) and in plasma. The inactive prodrug MLP is activated by thiolytic cleavage of its disulfide bridge releasing active MMC. In animal and human studies, plasma levels of non-liposomal MLP (protein-bound or free) and its cleavage product, MMC, are negligible or undetectable. This indicates that the prodrug remains non-cleaved and entrapped in circulating liposomes [11], enabling targeting of tumor tissue while decreasing systemic exposure to MMC. In tissues, MLP is cleaved by ubiquitous reducing agents abundant in tumors and other tissues [12–14], releasing locally active MMC [11].

A dose escalation phase 1A study of PL-MLP demonstrated lower toxicity and higher dose tolerability when compared to historical clinical data of MMC [9]. This study was expanded to a phase 1B to focus on advanced, chemo-refractory, colorectal cancer (CRC) patients and obtain safety, efficacy and pharmacokinetic (PK) data of PL-MLP as single agent and combined with either capecitabine (Cap) or Cap plus bevacizumab (Bev) in this specific patient population [15]. Here we report on the PK-analyzed CRC patient group (n = 53), the correlation of individual baseline clinical data with the PK parameters, and the correlation of individual PK data with survival and disease stabilization. These correlations reveal hitherto unknown associations or interactions that may help to optimize the clinical application of PL-MLP with possible implications for other liposome-based therapies.

Patients and methods

PL-MLP is a liposome product manufactured at Evonik Canada (Burnaby, BC) for Lipomedix Pharmaceuticals (Jerusalem, Israel). The active pharmaceutical ingredient, MLP, is synthesized at Laurus Labs. (Hyderabad, India). The product is supplied as a liposome suspension with a concentration of MLP of 5 mg/ml in 10-ml vials. The required dose is diluted in 250 ml physiologic saline bags and administered by intravenous infusion within 90–120 min.

[1] Promitil® is a registered trademark of Lipomedix Pharmaceuticals Ltd.

72 patients with metastatic CRC patients after failure to two or more lines of therapy received PL-MLP within the framework of a phase 1A-1B study [9, 15]. The pharmacokinetics of MLP was examined in 53 of these 72 patients who form the PK set patient cohort. Of note, one patient withdrew consent after cycle 1 and could not be evaluated for survival, although her PK data were available for analysis. PL-MLP was administered intravenously once every 4 weeks either as single agent (n = 28), in combination with Cap (n = 12), or in combination with Cap and Bev (n = 13). These combination cohorts were included in the study design primarily to investigate the impact of Cap and Bev, two common agents used in treatment of CRC, on the safety of PL-MLP. Cap was orally administered at a flat dose of 1000 mg bid from day 1 to 14, and Bev was intravenously administered at a dose of 5 mg/kg on days 1 and 15 in every 4-week cycle. Patients who reached study week 12 (3rd cycle) were evaluated for response by CT scan and Recist criteria.

The following baseline clinical parameters were analyzed for a correlation with survival and with the PK of MLP: age, sex, weight, body mass index (BMI), serum albumin, hemoglobin (Hgb), neutrophil, lymphocyte and platelet counts, and carcinoembryonic antigen (CEA) level.

The protocol was approved by the ethics committee at each participating institution and performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, and the Declaration of Helsinki. The trial was registered at clinical trials.gov with identifier NCT01705002. All patients signed informed consent.

PK analysis plan

PK analysis was performed on the PK analysis patient set (n = 53). Blood samples for analysis of plasma MLP levels were obtained prior and during the first 7 days after the 1st cycle of PL-MLP including the following post-end of infusion time points: 15 min, 1 h, 3 h, 6 h, 20–24 h, 44–48 h, 68–96 h, 7 days±1. PL-MLP infusion lasted approximately 90–120 min. Two plasma samples were analyzed per each time point and their arithmetic mean calculated. The plasma concentration of MLP for each time point was used for statistical analysis if at least 1 out of 2 values per time-point was above the lower limit of quantification (LLOQ). If both samples per time point were below LLOQ, they were considered as zero and ignored in the PK analysis. When one of the values per time point was above the LLOQ but the mean of both values was below LLOQ, it was reported as below the limit of quantification and ignored. Missing samples were ignored in the analysis. The actual sampling times were used for PK analysis.

The pharmacokinetic parameters were calculated with SAS© version 9.3 (or higher) software. The following pharmacokinetic parameters were calculated, using non-compartmental methods for plasma concentrations of MLP after single dose. The parameters included the following:

- Cmax (mg/L or μg/ml), maximum plasma concentration observed.
- $AUC_{o\text{-}t}$ (mg*h/L or μg*h/ml), area under the plasma concentration versus time curve calculated using the trapezoidal method from time zero to the actual time (t) corresponding to the last concentration above the limit of quantification (C).
- $AUC_{o\text{-}\infty}$ (mg*h/L or μg*h/ml), area under the plasma concentration versus time curve extrapolated to infinity according to the following equation: $AUCo\text{-}\infty = AUCo\text{-}t + (C_t/\lambda_z)$, where λ_z is elimination rate (Ke) or slope of the regression line of the terminal phase of the plasma concentration versus time curve.
- T½ (hours), terminal half-life determined according to the following equation: $T½ = 0.693/\lambda_z$.
- CL (L/h or ml/h), systemic clearance based on: CL = Dose / $AUC_{o\text{-}\infty}$.
- Vd (L or ml), apparent volume of distribution based on: Vd = Dose / λ_z * $AUC_{o\text{-}\infty}$.

Pharmacokinetic parameters of the PK patient set were summarized by arithmetic and geometric means, SEM, geometric SD factor or 95% CI, median, minimum, and maximum. For inter-dose comparisons and correlation analyses, Cmax and $AUC_{o\text{-}\infty}$ (simply referred to as AUC) were normalized to Cmax and AUC per mg of injected dose and abbreviated as Cmax/mg and AUC/mg.

MLP was extracted from plasma by ×10 fold dilution in isopropanol and measured by HPLC with UV detection at 360 nm wavelength as previously reported [11]. Mitomycin c levels were very low or undetectable and are not reported.

For statistical analysis, we used parametric and nonparametric (Mann-Whitney) *t* tests, linear regression and Pearson or Spearman correlation coefficients, and when appropriate log rank test and contingency analysis, all with Prism software, v. 8 (Graphpad, San Diego, CA). All *p* values are two-tailed.

Results

Patient characteristics (Table 1) All patients had advanced metastatic CRC and had been pretreated with 2 or more lines of chemotherapy for metastatic disease including fluopyrimidines, irinotecan, and oxaliplatin, and a significant fraction also received bevacizumab (92%) and/or anti-EGFR antibodies (38%). The MLP dose range was 0.5–3.5 mg/kg with most patients (55%) receiving 2.0–2.5 mg/kg. The median number of cycles administered per patient was 3 with a range between 1 and 12 (Table 2).

Safety The safety profile was similar to that observed in the phase 1A study [9]. No new safety signals were detected. Treatment was well tolerated particularly when the dose level was up to 2.0 mg/kg. The vast majority of Adverse Events (AEs) were mild to moderate in severity and unrelated to study

Table 1 Patient Characteristics of CRC patient set (Phase 1A and 1B)

Treatment Cohort							
Phase/ Cohort	1A Single Agent	1B Single Agent	1B Cap Combo[a]	1B Cap + Bev Combo	1B Bev Combo[b]	Whole CRC Patient Set	PK CRC Patient Set
N	11	17	23	13	8	72	53
Age (years)							
Mean (SD)	63.1(10.6)	65.2 (8.2)	61.4 (9.9)	61.0 (12.4)	54.3 (7.8)	61.7 (10.1)	63.7 (9.7)
Median	65.0	65.3	62.2	62.1	56.6	62.2	65.0
Min, Max	42.4, 76.1	51.3,78.6	40.4, 75.4	42.9, 83.5	39.6, 62.4	39.6, 83.5	42.4, 83.5
Sex N (%)							
Male	6 (55)	10 (59)	9 (61)	5 (38)	4 (50)	34 (47)	27 (51)
Female	5 (45)	7 (7)	14 (39)	8 (62)	4 (50)	38 (53)	26 (49)
ECOG N (%)							
0	5 (46)	8 (47)	16 (70)	7 (54)	6 (75)	42 (58)	29 (55)
1	4 (36)	9 (53)	6 (26)	6 (46)	2 (25)	27 (38)	22 (42)
2	2 (18)	1 (4)	3 (4)	2 (4)			

[a] In this group of patients, only 12 out of 23 participated in the PK study

[b] In this group, none of the 8 patients participated in the PK study

Table 2 Number of Cycles and Initial Dose of PL-MLP

Phase/ Cohort	1A Single Agent	1B Single Agent	1B Cap Combo[a]	1B Cap + Bev Combo	1B Bev Combo[b]	Whole CRC Patient Set	PK CRC Patient Set
N patients	11	17	23	13	8	72	53
Initial							
Dose (mg/kg)	0.5–3.5	3.0	2.0–2.5	2.0	2.0	0.5–3.5	0.5–3.5
N cycles							
Median	3.0	3.0	3.0	3.0	3.5	3.0	3.0
Min	1	1	1	1	2	1	1
Max	12	7	8	6	6	12	12

[a] In this cohort, only 12 out of 23 participated in the PK study

[b] In this cohort, none of the 8 patients participated in the PK study

treatment. At higher dose levels, fatigue and loss of appetite were more frequent. Dose reductions were seldom required. As previously reported, thrombocytopenia was the main dose-limiting factor upon cumulative dosing [9, 15].

No deaths were related to study treatment. Life-threatening events were reported by ten patients. With the exception of one report of thrombocytopenia certainly related to the study treatment, all other life-threatening events were considered unrelated or unlikely to be related to the study treatment. Among all 72 CRC patients, two Grade 3 thrombocytopenia events (probably related) and one Grade 3 fatigue (possibly related) were considered dose-limiting toxicities and patient participation in study was subsequently discontinued. Of the 50 reported SAE's, only two were considered possibly related to study treatment. The remainder were unlikely related or unrelated. Low platelet count, fatigue and anemia were the most frequently reported SAE's.

Based on the safety analysis (Data on file, Lipomedix Pharmaceuticals Inc.), there was no apparent added toxicity in patients receiving PL-MLP in combination with Capecitabine and/or Bevacizumab.

Response and survival 36 patients of the PK analysis patient set received 3 or more cycles of PL-MLP and were evaluable for efficacy response on week 12. Stable Disease (SD) was the best response observed in 42% (15/36) of the evaluable patients and in 29% (15/52) of the whole PK patient set. Survival data for the various patient groups are presented in Table 3.

Median survival of all patients ($n = 52$) and of evaluable patients ($n = 36$) was 6.4 months and 8.7 months, respectively. The median survival times of SD patients (14.4 months), progressive disease (PD) patients (6.5 months), and early drop-out/non-evaluable (NE) patients (2.3 months) were significantly different (Fig. 1) and substantially longer in SD patients. There was also a major difference (>4-fold) in the fraction of 1-year survivors when the SD patient group was compared to other groups (Table 3). The survival and disease stabilization rates of the PK patient set ($n = 52$) were similar to the data for the whole CRC patient data set ($n = 71$) (data not shown).

The addition of Cap and/or Bev to PL-MLP treatment and the effect of the start dose of MLP on survival and disease stabilization were examined (Fig. 2a-b). Cap and Bev did not have any apparent effect on survival (Fig. 2a), nor on the rate of SD (not shown). To examine the effect of PL-MLP dose, patients were ranked by their starting dose and divided in 2 groups of 26 patients each (0.5–2.0 and 2.5–3.5 mg/kg). As seen in Fig. 2b, there was a possible deleterious effect on survival of the higher dose level (median survival: 9.6 and 5.0 months for low and high start dose groups respectively, $p = 0.0077$), suggesting that any increase of dose above 2.0 mg/kg may contribute to toxicity and has no added therapeutic value. The rate of SD in the low start-dose group (10/26) and in the high start-dose group (5/26) were not significantly different (Fisher's exact test).

Table 3 Survival of the PK patient set by response group

Patient Group	All	Evaluable[a]	SD	PD	NE
N patients	52	36	15	21	16
Median (months)	6.4	8.7	14.4	6.5	2.3
95%CI[b]	4.5–8.8	6.5–12.3	9.6–19.3	4.6–8.8	1.6–3.4
%1-year survival (N/Total)	23.1% (12/52)	33.3% (12/36)	60.0% (9/15)	14.3% (3/21)	0 (0/16)

[a] Evaluable includes all SD and PD patients alive and on study in week 12

[b] The actual CI of the median survival values ranged between 96% and 98%

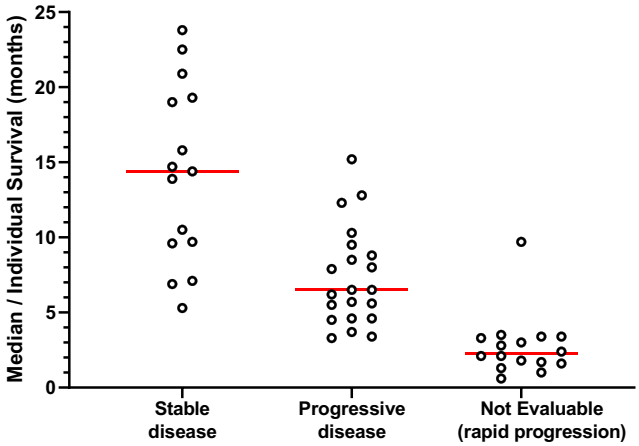

Fig. 1 Survival per patient response group. Based on the PK Analysis Patient Set ($n = 52$): SD vs PD, $p < 0.0001$ (*t* test), $p = 0.0002$ (Mann-Whitney); PD vs NE, $p < 0.0001$ (both *t* and Mann-Whitney tests)

PK parameters The median, mean, and geometric mean values for Cmax, AUC, T½, CL, and Vd with normalization of Cmax and AUC per mg of injected dose are presented in Table 4 as the average of all dose levels and in the Electronic Supplementary Material (ESM)-Table 1 per dose level group. The PK of MLP was stealth-like with a long T½, just under 24 h, slow CL, and a small Vd just slightly above the estimated plasma patient volume. The plasma time-concentration elimination curves of MLP in 3 representative patients are presented in Fig. 3. There was inter-patient variability particularly in the T½ values even at the same dose of MLP. For example, at 2 mg/kg the range of T½ was 13 to 41 h, more than a 3-fold difference. The MLP start dose had a significant effect on the PK with a trend to higher Cmax/mg dose and AUC/mg dose and lower CL and Vd when the dose is gradually raised, particularly from 2.0 to 3.0 mg/kg (ESM-Fig. 1). This is consistent with prior observations in the phase 1A study of PL-MLP in which a trend to clearance saturation was noticed at doses above 2.0 mg/kg [9].

Correlation of baseline clinical parameters with survival For this analysis, we used the whole 71 CRC patient set for whom survival data were available. Baseline BMI, serum albumin, and Hgb levels were positively correlated with survival while baseline CEA, neutrophil, and platelet counts were negatively correlated with survival (ESM-Fig. 2). Age, sex, weight, and lymphocyte count were not correlated with survival in this patient data set.

Correlation of baseline clinical parameters with PK parameters We looked at a possible association between various patient pre-treatment clinical factors and the PK parameters of MLP. We found that a high neutrophil count and a high CEA baseline were significantly associated with low Cmax/mg and AUC/mg, faster CL, larger Vd, and shorter T½ (only for neutrophil count), suggesting a direct or indirect relationship of these factors with the disposition and/or distribution of liposomal MLP from circulation. The strongest correlations (Pearson $r > 0.50$) were found between neutrophil count and clearance and between CEA level and Vd (Fig. 4). Pearson r values for other significant correlations not shown in Fig. 4 are: neutrophil count with Cmax/mg, $r = -0.32$ ($p = 0.020$), with AUC/mg, $r = -0.37$ ($p = 0.0068$), and with T½, $r = -0.40$ ($p = 0.0028$); CEA level with Cmax/mg: $r = -0.44$ ($p = 0.0012$), and with AUC/mg, $r = -0.56$ ($p < 0.0001$).

Other factors (sex, age, weight, BMI, albumin, Hgb, platelet count, lymphocyte count) did not show any significant correlation with the PK parameters of MLP.

Correlation of PK parameters with survival and response We then examined whether there is an association of the PK parameters with response and survival. Longer T½, greater AUC/mg dose, and slower CL were significantly correlated with longer survival (Fig. 5 and inset table). Notably, all 12 patients with survival >1 year had T½ > 20 h. No such correlation was found for Cmax/mg dose and Vd. In addition, we

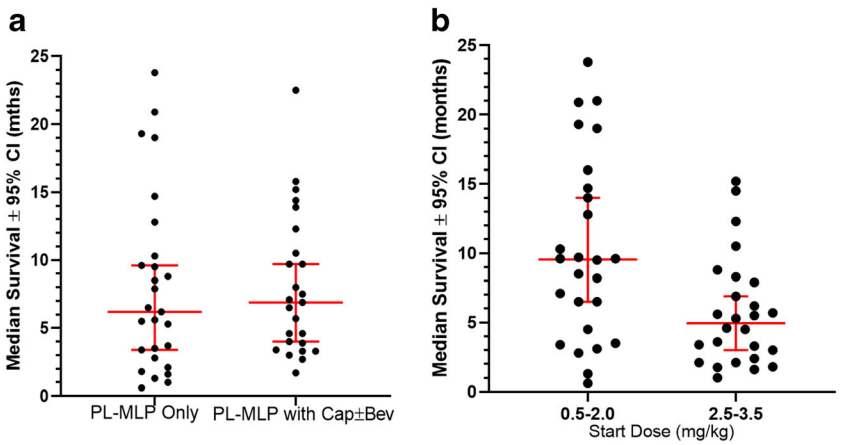

Fig. 2 Survival of PL-MLP-treated patients -Effects of combination with Cap ± Bev and Start Dose level. a. PL-MLP only ($n = 27$) vs. PL-MLP with Cap±Bev ($n = 25$), not significant (Mann-Whitney test). **b**. Start Dose 0.5–2.0 mg/kg ($n = 26$) vs. Start Dose 2.5–3.5 mg/kg ($n = 26$), median 9.6 months vs. 5.0 months, $p = 0.0077$ (Mann-Whitney test)

Table 4 PK Parameters of MLP across all dose levels (n = 53)

Parameter	Cmax/mg dose	AUC/mg dose	T½	CL	Vd
Units	mg/L	mg*h/L	h	ml/h	L
Geometric Mean	0.28	7.14	21.3	115	3.52
95% CI	0.26–0.31	6.28–8.105	19.8–23.0	100–130	3.21–3.86
Mean	0.29	7.90	22.1	130	3.72
SEM	0.01	0.54	0.8	10	0.18

observed a significantly longer T½ and slower CL in the SD patient group as compared to the PD and NE patient group (Fig. 6a-b).

Discussion

The treatment of advanced CRC patients refractory to oxaliplatin, irinotecan, fluopyrimidines, and, in the case of Ras-wild type tumors, to anti-EGFR antibodies remains very disappointing as reflected by the minimal impact on survival of two new drugs (Regorafenib, TAS-102) approved for this indication in the last decade [16, 17] and the restricted activity of anti-PD1 antibodies to a very small fraction of patients with high MSI at this advanced stage [18].

MMC is a powerful cytotoxic and antibacterial agent with activity against a broad spectrum of tumors including multi-drug resistant (MDR) tumor and stem cells, but also with problematic toxicities limiting its systemic use in the clinic at effective pharmacologic doses [19]. MMC has been used in metastatic CRC, particularly in combination with fluopyrimidines, and found to be an effective agent in 1st and 2nd or 3rd line chemotherapy [20, 21]. A review of clinical studies with MMC in CRC concludes that the drug has modest efficacy in patients refractory to standard treatment and is a valid approach as alternative to supportive care [22]. A more recent study has shown that the combination of bevacizumab, capecitabine and MMC is safe and more effective than capecitabine with regard to PFS and response rate in 1st line chemotherapy [23], with an additional survival benefit in the elderly subpopulation of the study [24].

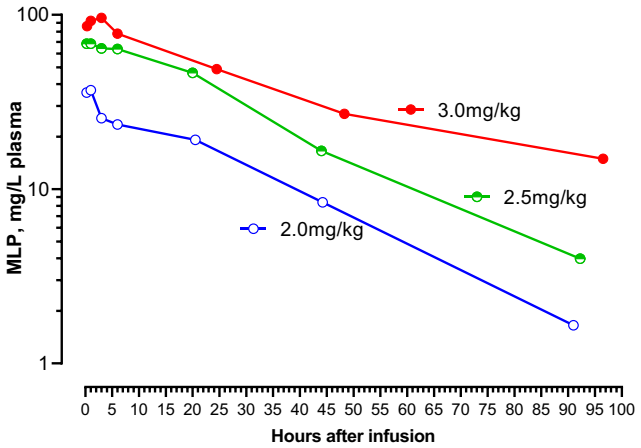

Fig. 3 PK clearance curves of MLP curves in 3 representative CRC patients. Plasma clearance curves of MLP are nearly mono-exponential. The effect of dose on plasma levels is clearly noticeable

However, in most clinical studies, the dose of MMC has been limited to a very low dose intensity of approximately 1.2 mg/m^2/week (7 mg/m^2 q 6 week) and to a very low cumulative dose of 28 mg/m^2 for fear of toxicity. These are much lower doses than the achievable doses with PL-MLP in MMC-equivalents, which are ~7.0 mg/m^2/week for single dose cycle (2.5 mg/kg q 4 week) and ~140 mg/m^2 cumulative dose[2] [9]. In addition to this major advantage in dose safety, an additional factor that may confer a pharmacologic advantage to PL-MLP is the EPR effect [5] which results in passive tumor targeting of long-circulating and stable liposomal formulations and enhanced tumor exposure to the drug payload [11]. The disease stabilization rates obtained with PL-MLP in this study at week 12 (29% for all patients and 42% for the evaluable patients) compare favorably with historical phase 3 data for Regorafenib (19%), TAS-102 (23%), and Placebo-treated (4%) patients in a similar disease stage [16, 17], suggesting that PL-MLP may have significant added value in the management of advanced CRC. The favorable safety profile of PL-MLP with mild myelosuppression, no gastrointestinal toxicity, no skin toxicity and no neurotoxicity may well allow combination with old (capecitabine) or newer fluopyrimidines (TAS-102), with various biological agents approved for treatment of CRC (anti-EGFR and anti-VEGF/VGEFR2 antibodies), and even with irinotecan given the potentiating effect of MMC on DNA Topo-isomerase I inhibitors [25].

While many PK studies have been reported with various nanomedicines, few have addressed the correlation of the PK parameters with response and survival. Understanding these correlations is important to determine what makes one

[2] Calculated for an MLP single dose of 2.5 mg/kg q4weeks and a cumulative MLP dose of 12.5 mg/kg (1 cycle of 2.5 mg/kg and 5 cycles of 2 mg/kg) to an adult patient of 70 kg weight and 170 cm height. Conversion factor: 1 mg MMC = 3.4 mg MLP.

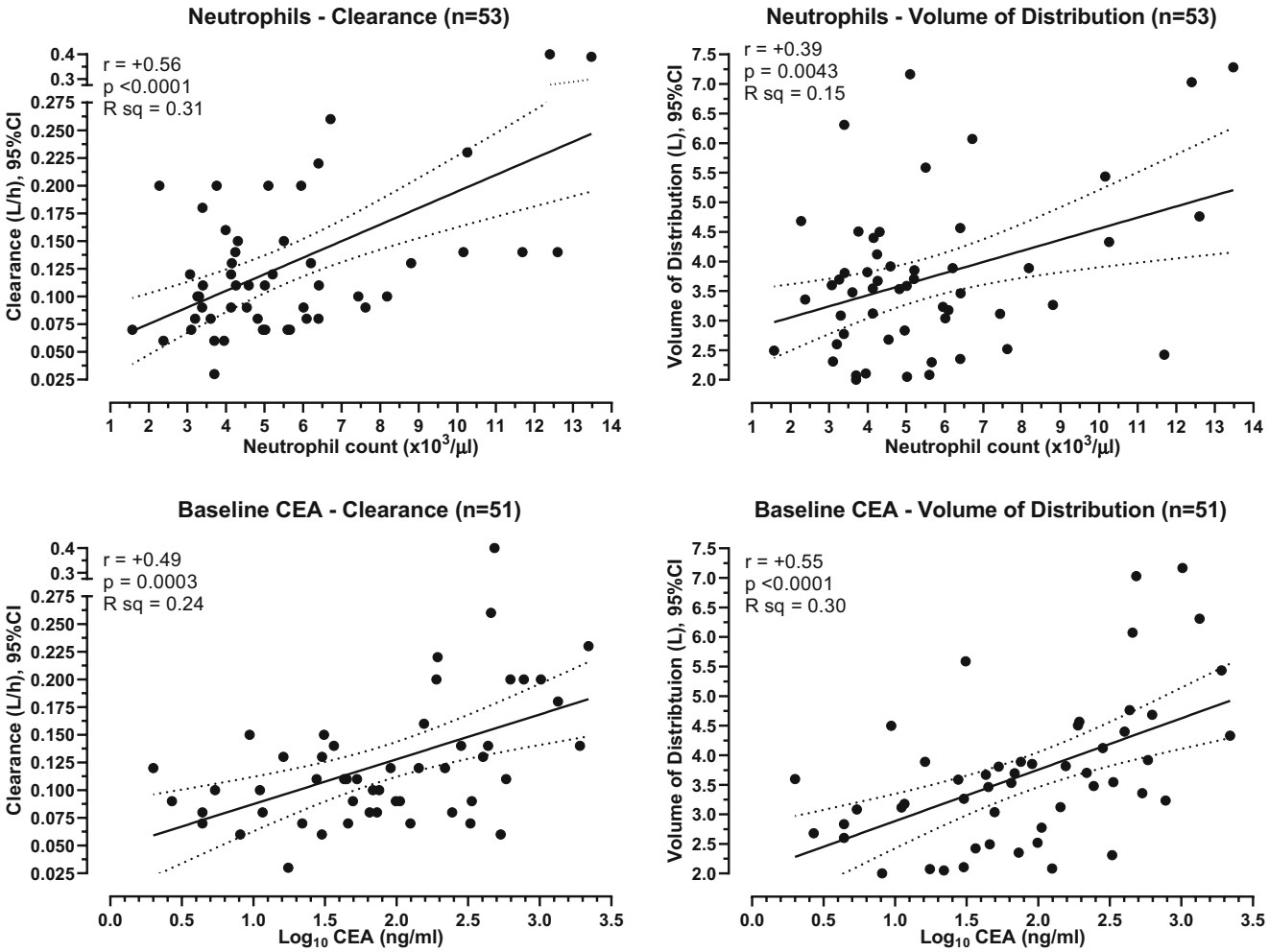

Fig. 4 Correlation of baseline Neutrophil count and CEA level with Clearance and Volume of Distribution. r = Pearson Correlation Coefficient; R sq. = Goodness of linear regression fit (r^2)

nanomedicine work better than others and may help to optimize the clinical application of PL-MLP and other liposome-based therapies.

The longer circulation time of the liposomal prodrug in SD patients and its correlation with longer survival are consistent with the well-known correlation between liposome circulation time and localization in tumors. The correlation of high neutrophil counts with shorter survival is not surprising, as many of these myeloid derived cells are immunosuppressive, but the correlation with faster CL of a liposomal drug, as shown here for PL-MLP, is a novel observation. A high neutrophil count may be a sign of inflammatory macrophage activation resulting in shorter liposomal circulation half-life and reducing the chances of disease control.

A high CEA level generally predicts a poor prognosis and short survival. Serum CEA is one of the most widely used tumor markers for assessing treatment response and detecting recurrence in CRC. CEA is part of a family of adhesion glycoprotein molecules that serve as functional L-selectin and E-selectin ligands of colon carcinoma cells, and may be critical to their metastatic dissemination [26, 27]. Yet, the correlation of a higher CEA with larger Vd and faster CL was rather unexpected and may be an indirect indication of greater vascular permeability and larger extracellular fluid volume related to high CEA levels and/or other co-elevated inflammatory cytokines in this group of poor prognosis patients.

Contrary to the frequently retarded clearance of many low molecular weight drugs in patients with advanced disease and worse baseline clinical parameters, we found that PL-MLP is cleared faster in these patients. This underscores another important difference between nanomedicines and conventional drugs with clinical implications. Probes assessing the mononuclear phagocyte system activity for predicting the pharmacokinetics and pharmacodynamics of liposomal drugs have been proposed, and a correlation of pre-treatment monocyte counts with liposomal drug clearance in humans has been reported [28–30].

Correlation with Survival	Half-life (T½)	AUC/mg dose	Clearance
Spearman r	+0.42	+0.30	-0.32
p value	0.0020	0.0308	0.0222

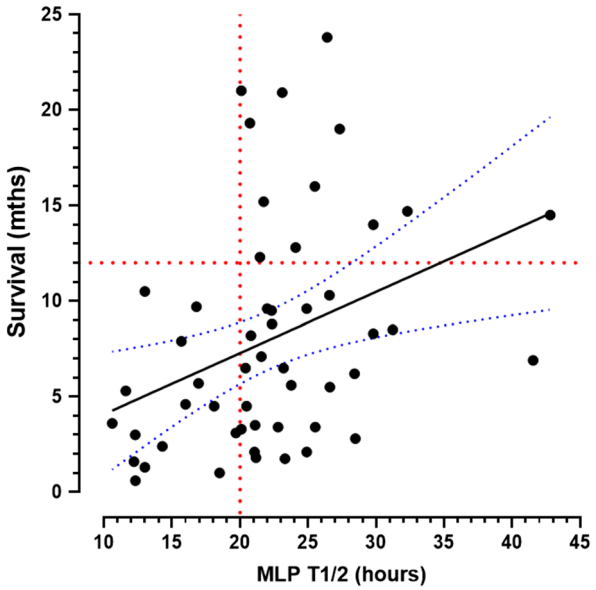

Fig. 5 PK of MLP and Survival. All 12 patients with survival >12 months (above dashed line) had T½ > 20 h. Inset Table shows that T½, as well as AUC/mg, and Clearance of MLP are significantly correlated with Survival

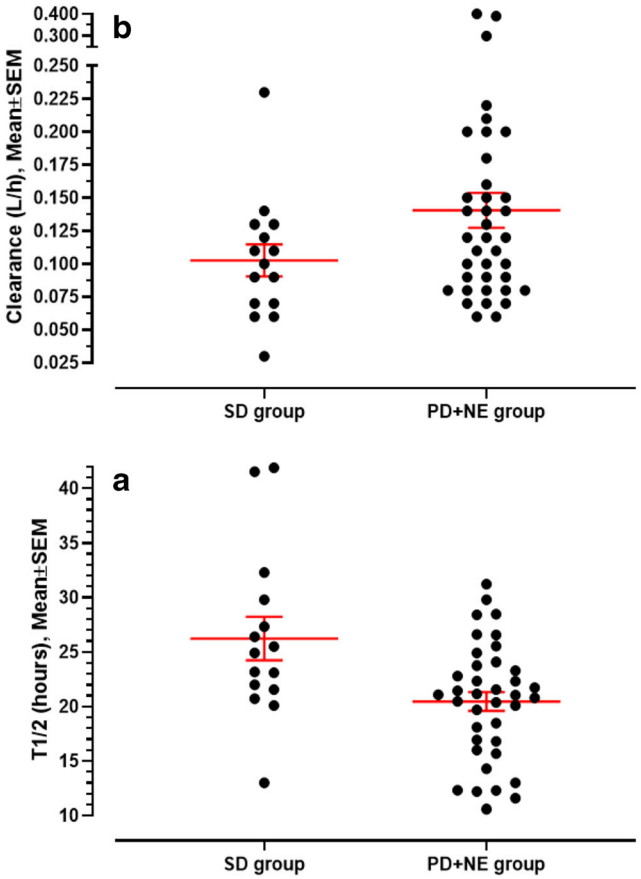

Fig. 6 PK parameters of MLP and disease stabilization. a: Mean T½: SD group = 26.2 h, PD + NE group = 20.5 h, $p = 0.0031$ (*t* test).; **b**: Mean Clearance: SD group = 103 ml/h, PD + NE group = 141 ml/h, $p = 0.0396$ (*t* test). SD group, $n = 15$; PD + NE group, $n = 38$

Furthermore, the association of the long circulation time of liposomal MLP with SD and with longer survival is consistent with an improved probability of disease control resulting from enhanced tumor localization of long-circulating liposomes and underscores the relevance of personalized pharmacokinetic evaluation in the clinical use of nanomedicines [31]. The correlation found between some key PK parameters of MLP on the one hand, and survival on the other hand, suggests that the PK of MLP during the 1st cycle of PL-MLP therapy can help predict the patient chances to achieve disease stabilization and to survive longer than the expected historical average. Earlier studies with pegylated liposomal doxorubicin had also indicated significant pharmacokinetic-pharmacodynamic relationships vis-à-vis toxicity [32] and efficacy [33].

While we recognize that the joint analysis of different patient cohorts receiving single agent PL-MLP or combined treatment of PL-MLP with Cap and/or Bev may compromise the interpretation of our observations, there was no indication that Cap and/or Bev affected the PK of PL-MLP and it seems unlikely that these agents could have a significant contribution to tumor control and survival given the prior exposure of most of the study patients to both agents.

Finally, our observations also raise the possibility of direct intervention by blocking inflammation (COX-2 inhibitors, anti-IL1 antibodies, NF kappa B blockers, other) or suppressing tumor-associated macrophages [34] along with PL-MLP treatment or treatment with other nanomedicines to further improve their PK and thereby their therapeutic efficacy.

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Compliance with ethical standards

Conflict of interest A. Gabizon is founder, chief scientist and a director of Lipomedix; E. Tahover declares that she has no conflict of interest; T. Golan declares that she has no conflict of interest; R. Geva declares that she has no conflict of interest; R. Perets declares that she has no conflict of interest; Y. Amitay is an employee (scientist) of Lipomedix; H. Shmeeda declares that she has no conflict of interest; P. Ohana is an employee (vice-president) of Lipomedix.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the

institutional review boards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Cattel L, Ceruti M, Dosio F (2004) From conventional to stealth liposomes: a new frontier in cancer chemotherapy. J Chemother (Florence, Italy) 16(Suppl 4):94–97. https://doi.org/10.1179/joc.2004.16.Supplement-1.94
2. Allen TM, Cullis PR (2013) Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev 65(1):36–48. https://doi.org/10.1016/j.addr.2012.09.037
3. Gabizon AA, Patil Y, La-Beck NM (2016) New insights and evolving role of pegylated liposomal doxorubicin in cancer therapy. Drug Resist Updat 29:90–106. https://doi.org/10.1016/j.drup.2016.10.003
4. Glassman DC, Palmaira RL, Covington CM, Desai AM, Ku GY, Li J, Harding JJ, Varghese AM, O'Reilly EM, Yu KH (2018) Nanoliposomal irinotecan with fluorouracil for the treatment of advanced pancreatic cancer, a single institution experience. BMC Cancer 18(1):693. https://doi.org/10.1186/s12885-018-4605-1
5. Prabhakar U, Maeda H, Jain RK, Sevick-Muraca EM, Zamboni W, Farokhzad OC, Barry ST, Gabizon A, Grodzinski P, Blakey DC (2013) Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. Cancer Res 73(8):2412–2417. https://doi.org/10.1158/0008-5472.can-12-4561
6. Gabizon AA, Tzemach D, Horowitz AT, Shmeeda H, Yeh J, Zalipsky S (2006) Reduced toxicity and superior therapeutic activity of a mitomycin C lipid-based prodrug incorporated in pegylated liposomes. Clin Cancer Res 12(6):1913–1920
7. Zalipsky S, Saad M, Kiwan R, Ber E, Yu N, Minko T (2007) Antitumor activity of new liposomal prodrug of mitomycin C in multidrug resistant solid tumor: insights of the mechanism of action. J Drug Target 15(7-8):518–530. https://doi.org/10.1080/10611860701499946
8. Gabizon A, Amitay Y, Tzemach D, Gorin J, Shmeeda H, Zalipsky S (2012) Therapeutic efficacy of a lipid-based prodrug of mitomycin C in pegylated liposomes: studies with human gastro-entero-pancreatic ectopic tumor models. J Control Release 160(2):245–253. https://doi.org/10.1016/j.jconrel.2011.11.019
9. Golan T, Grenader T, Ohana P, Amitay Y, Shmeeda H, La-Beck NM, Tahover E, Berger R, Gabizon AA (2015) Pegylated liposomal mitomycin C prodrug enhances tolerance of mitomycin C: a phase 1 study in advanced solid tumor patients. Cancer Med 4(10):1472–1483. https://doi.org/10.1002/cam4.491
10. Tahover E, Bar-Shalom R, Sapir E, Pfeffer R, Nemirovsky I, Turner Y, Gips M, Ohana P, Corn BW, Wang AZ, Gabizon AA (2018) Chemo-radiotherapy of Oligometastases of colorectal Cancer with Pegylated liposomal Mitomycin-C Prodrug (Promitil): mechanistic basis and preliminary clinical experience. Front Oncol 8:544. https://doi.org/10.3389/fonc.2018.00544
11. Amitay Y, Shmeeda H, Patil Y, Gorin J, Tzemach D, Mak L, Ohana P, Gabizon A (2016) Pharmacologic studies of a Prodrug of Mitomycin C in Pegylated liposomes (Promitil((R))): high stability in plasma and rapid Thiolytic Prodrug activation in tissues. Pharm Res 33(3):686–700. https://doi.org/10.1007/s11095-015-1819-7
12. Arner ES, Holmgren A (2006) The thioredoxin system in cancer. Semin Cancer Biol 16(6):420–426. https://doi.org/10.1016/j.semcancer.2006.10.009
13. Soderberg A, Sahaf B, Rosen A (2000) Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in human plasma. Cancer Res 60(8):2281–2289
14. Powis G, Kirkpatrick DL (2007) Thioredoxin signaling as a target for cancer therapy. Curr Opin Pharmacol 7(4):392–397. https://doi.org/10.1016/j.coph.2007.04.003
15. Gabizon A, Grenader T, Tahover E, Shmeeda H, Golan T, Berger R, Geva R, Wolf I, Perets R, Amitay Y, Ohana P (2016) ESMO 2016 congress abstract: a phase 1B study of pegylated liposomal mitomycin-C prodrug with or without capecitabine and bevacizumab in third line chemotherapy of colorectal cancer. Ann Oncol 27(6):149–206. https://doi.org/10.1093/annonc/mdw370
16. Mayer RJ, Van Cutsem E, Falcone A, Yoshino T, Garcia-Carbonero R, Mizunuma N, Yamazaki K, Shimada Y, Tabernero J, Komatsu Y, Sobrero A, Boucher E, Peeters M, Tran B, Lenz HJ, Zaniboni A, Hochster H, Cleary JM, Prenen H, Benedetti F, Mizuguchi H, Makris L, Ito M, Ohtsu A, Group RS (2015) Randomized trial of TAS-102 for refractory metastatic colorectal cancer. N Engl J Med 372(20):1909–1919. https://doi.org/10.1056/NEJMoa1414325
17. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouche O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D, Group CS (2013) Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 381(9863):303–312. https://doi.org/10.1016/S0140-6736(12)61900-X
18. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr (2015) PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 372(26):2509–2520. https://doi.org/10.1056/NEJMoa1500596
19. Verweij J, Pinedo HM (1990) Mitomycin C: mechanism of action, usefulness and limitations. Anti-Cancer Drugs 1(1):5–13
20. Chong G, Dickson JL, Cunningham D, Norman AR, Rao S, Hill ME, Price TJ, Oates J, Tebbutt N (2005) Capecitabine and mitomycin C as third-line therapy for patients with metastatic colorectal cancer resistant to fluorouracil and irinotecan. Br J Cancer 93(5):510–514. https://doi.org/10.1038/sj.bjc.6602733
21. Rao S, Cunningham D, Price T, Hill ME, Ross PJ, Tebbutt N, Norman AR, Oates J, Shellito P (2004) Phase II study of capecitabine and mitomycin C as first-line treatment in patients with advanced colorectal cancer. Br J Cancer 91(5):839–843. https://doi.org/10.1038/sj.bjc.6602039
22. Dimou A, Syrigos KN, Saif MW (2010) Is there a role for mitomycin C in metastatic colorectal cancer? Expert Opin Investig Drugs 19(6):723–735. https://doi.org/10.1517/13543784.2010.485191
23. Tebbutt NC, Wilson K, Gebski VJ, Cummins MM, Zannino D, van Hazel GA, Robinson B, Broad A, Ganju V, Ackland SP, Forgeson G, Cunningham D, Saunders MP, Stockler MR, Chua Y, Zalcberg JR, Simes RJ, Price TJ (2010) Capecitabine, bevacizumab, and mitomycin in first-line treatment of metastatic colorectal cancer: results of the Australasian gastrointestinal trials group randomized phase III MAX study. J Clin Oncol 28(19):3191–3198. https://doi.org/10.1200/JCO.2009.27.7723
24. Price TJ, Zannino D, Wilson K, Simes RJ, Cassidy J, Van Hazel GA, Robinson BA, Broad A, Ganju V, Ackland SP, Tebbutt NC (2012) Bevacizumab is equally effective and no more toxic in elderly patients with advanced colorectal cancer: a subgroup analysis from the AGITG MAX trial: an international randomised controlled trial of Capecitabine, Bevacizumab and Mitomycin C. Ann Oncol 23(6):1531–1536. https://doi.org/10.1093/annonc/mdr488

25. Xu Y, Kolesar JM, Schaaf LJ, Drengler R, Duan W, Otterson G, Shapiro C, Kuhn J, Villalona-Calero MA (2009) Phase I and pharmacokinetic study of mitomycin C and celecoxib as potential modulators of tumor resistance to irinotecan in patients with solid malignancies. Cancer Chemother Pharmacol 63(6):1073–1082. https://doi.org/10.1007/s00280-008-0826-3
26. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K (2008) Carcinoembryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow. J Biol Chem 283(23):15647–15655. https://doi.org/10.1074/jbc.M800543200
27. Konstantopoulos K, Thomas SN (2009) Cancer cells in transit: the vascular interactions of tumor cells. Annu Rev Biomed Eng 11:177–202. https://doi.org/10.1146/annurev-bioeng-061008-124949
28. Giovinazzo H, Kumar P, Sheikh A, Brooks KM, Ivanovic M, Walsh M, Caron WP, Kowalsky RJ, Song G, Whitlow A, Clarke-Pearson DL, Brewster WR, Van Le L, Zamboni BA, Bae-Jump V, Gehrig PA, Zamboni WC (2016) Technetium Tc 99m sulfur colloid phenotypic probe for the pharmacokinetics and pharmacodynamics of PEGylated liposomal doxorubicin in women with ovarian cancer. Cancer Chemother Pharmacol 77(3):565–573. https://doi.org/10.1007/s00280-015-2945-y
29. Caron WP, Lay JC, Fong AM, La-Beck NM, Kumar P, Newman SE, Zhou H, Monaco JH, Clarke-Pearson DL, Brewster WR, Van Le L, Bae-Jump VL, Gehrig PA, Zamboni WC (2013) Translational studies of phenotypic probes for the mononuclear phagocyte system and liposomal pharmacology. J Pharmacol Exp Ther 347(3):599–606. https://doi.org/10.1124/jpet.113.208801
30. La-Beck NM, Zamboni BA, Gabizon A, Schmeeda H, Amantea M, Gehrig PA, Zamboni WC (2012) Factors affecting the pharmacokinetics of pegylated liposomal doxorubicin in patients. Cancer Chemother Pharmacol 69(1):43–50. https://doi.org/10.1007/s00280-011-1664-2
31. Grodzinski P, Kircher M, Goldberg M, Gabizon A (2019) Integrating nanotechnology into Cancer care. ACS Nano 13(7):7370–7376. https://doi.org/10.1021/acsnano.9b04266
32. Lyass O, Uziely B, Ben-Yosef R, Tzemach D, Heshing NI, Lotem M, Brufman G, Gabizon A (2000) Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in metastatic breast carcinoma. Cancer 89(5):1037–1047. https://doi.org/10.1002/1097-0142(20000901)89:5<1037::AID-CNCR13>3.0.CO;2-Z
33. Amantea MA, Forrest A, Northfelt DW, Mamelok R (1997) Population pharmacokinetics and pharmacodynamics of pegylated-liposomal doxorubicin in patients with AIDS-related Kaposi's sarcoma. Clin Pharmacol Ther 61(3):301–311. https://doi.org/10.1016/S0009-9236(97)90162-4
34. Rajan R, Sabnani MK, Mavinkurve V, Shmeeda H, Mansouri H, Bonkoungou S, Le AD, Wood LM, Gabizon AA, La-Beck NM (2018) Liposome-induced immunosuppression and tumor growth is mediated by macrophages and mitigated by liposome-encapsulated alendronate. J Control Release 271:139–148. https://doi.org/10.1016/j.jconrel.2017.12.023