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Lycopene minimizes skin toxicity and oxidative stress in patients treated with panitumumab-containing therapy for metastatic colorectal cancer

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ABSTRACT

Epidermal Growth Factor Receptor (EGFR) inhibition leads to the production of reactive oxygen metabolites causing skin inflammatory reactions. Anti-EGFR therapies are frequently associated with skin toxicities which often cause treatment delay and impairment to patient quality of life. Lycopene is a compound in the carotenoid group with an extreme antioxidant action which accumulates in the skin due to its hydrophobic structure. In a pilot study, we describe lactolycopene effectiveness in reducing skin toxicity and protecting tissues from oxidative stress in patients treated with panitumumab. Despite the limited number of patients, we show an absolute reduction of skin grade 2–3 toxicity in 41% of patients and 46% of panitumumab cumulative cycles in the experimental group versus placebo; lactolycopene administration was able to abolish malondialdehyde (MDA) production, a biomarker used to measure lipid peroxidation in the organism, and replenish antioxidant consumption in the course of anti-EGFR therapy.

Trial registration: Clinicaltrials.gov NCT 03,167,268 (Pasto trial).

Abbreviations: Folfiri, leukovorin, 5-fluorouracil, and irinotecan; Folfox, leukovorin, 5-fluorouracil, and oxaliplatin; ROS, reactive oxygen species; EGFR, Epidermal growth factor receptor; MDA, malondialdehyde.

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1. Introduction

Anti-neoplastic agents induce the production of reactive oxygen species (ROS) in patients treated for neoplastic diseases (Weijl NI et al., 1997). Moreover, Epidermal Growth Factor Receptor (EGFR) inhibition leads to the production of ROS, particularly causing skin inflammatory reactions. ROS injure cells and tissue directly via oxidative degradation of essential cellular components and indirectly by altering the protease/antiprotease balance that normally exists within the interstitial space of the tissue. ROS may also initiate and/or amplify inflammation via the upregulation of different genes involved in the inflammatory response by the activating transcription factors, such as nuclear transcription factor kB (NF-kB) (Weijl NI et al.,1997; Busam KJ et al., 2001; Ben-Dor A et al., 2005). Most patients treated with anti-EGFR drugs show dermatologic toxicity of any grade, and 10–20% of them with severe toxicity which can cause treatment delay and impairment to patient quality of life (Van Custen E et al., 2007).

Lycopene is a compound in the carotenoid group, largely contained in tomatoes and their derivatives, with an extreme antioxidant action. Tomato (*Solanum Lycopersicum*) is the biggest food source but lycopene is contained in an array of other pink or red fruits as well as guava (*Psidium Guajava*), watermelon (*Citrullus Lanatus*), papaya (*Carica Papaya*), pink grapefruit *(Citrus Paradisi)* and others. In the pericarpal tissue of ripe tomato fruits, lycopene is localized in cellular compartments, the chromoplasts, where the crystals are associated with membrane structures. The characteristics of a lycopene-based formulation have a marked effect on the bioavailability of lycopene, including complexation with proteins, minimized crystal size, and cis- rather than trans- isomer. The bioavailability of lycopene from tomato products is greatly enhanced after mechanical texture disruption and thermal processing. Lycopene from fresh and unprocessed tomatoes is poorly absorbed by humans whereas absorption is higher from processed foods such as tomato paste and tomato juice heated in oil. Similar bioavailability is exhibited by “lactolycopene”, a formulation in which lycopene from tomato oleoresin is embedded in a whey protein matrix (Conner EM Grisham MB, 1996; Di Mascio P et al., 1989; Richelle M et al., 2002). As a result of its hydrophobic property, lycopene accumulates specifically in the skin and, as a powerful ROS scavenger, it may well be effective in reducing anti-EGFR drug toxicity at this site (Young AJ Lowe GM, 2001; Balić A Mokos M, 2019).

2. Materials and methods

2.1. Study design and patients

This multi-centre phase-II randomized double-blind Panitumumab Skin Toxicity Prevention (PASTO) pilot study enrolled patients with metastatic colorectal cancer undergoing panitumumab-containing therapy. In total, 28 patients were treated with lactolycopene or placebo p.o. in order to assess the effectiveness of prophylactic treatment with lactolycopene in reducing skin toxicity and oxidative stress. Standard schedules of panitumumab containing therapy were administered according to therapeutic indications as a single agent or in combination with Oxaliplatin, Irinotecan and Fluorouracil every two weeks. Main inclusion criteria included: a) no previous treatment with anti-EGFR drugs, b) no other systemic antineoplastic therapy during the three weeks before randomization, c) no dermatological ongoing diseases which contraindicated the treatment or made skin toxicity assessment difficult, d) absence of clinical conditions which could alter lycopene absorption (altered intestinal transit, malabsorption) and e) no intolerance/allergy to tomato or milk.

Written informed consent for experimentation with human subjects was obtained from all patients. The protocol was approved by the ethics committees of all the participating institutions.

2.2. Randomization and masking

Patients were randomly assigned (1:1) to lactolycopene or placebo treatment with a computerized minimization algorithm that generated a trial number and drug pack allocation for each patient. Randomization was done centrally by the Clinical Epidemiology and Biometrics Service – Scientific Direction, IRCCS Foundation San Matteo Hospital, Pavia. Sequence generation, enrolment and trial group assignment were carried out by the statistician who was not involved in the clinical running of the trial or data collection. Neither patients nor researchers were aware of the treatment given to each patient. The two groups were balanced according to gender, the administration of panitumumab in monotherapy or in association with chemotherapy and the institution where patients were treated. Identical looking lactolycopene and whey-proteins-containing placebo tablets were prepared specifically for the trial, packaged and labelled in identical blister packs by Indena Pharmaceutical (Indena S.p.A., Milan, Italy) for each patient, and specifically distributed to institutions according to the randomization list. Patients were enrolled by research staff at each centre after undergoing eligibility checks.

2.3. Procedures

Administration of a daily 20 mg dose or lactolycopene or placebo commenced the day before the first cycle of panitumumab and was taken orally for the entire duration of the treatment, until progression of the disease or definitive drug suspension for toxicity. Standard supportive care, comprising moisturizing cream, topical use of antibiotics and/or hydrocortisone, could be administered to patients according to clinical practice. The administration of tetracyclines p.o., frequently used to treat papulopustular eruption during anti-EGFR treatments, was not allowed for a prophylactic purpose but only for skin toxicity $\geq$ G2.

Skin toxicity was evaluated in accordance with the Multinational Association for Supportive Care in Cancer (MASCC) Criteria Score for skin toxicity due to anti-EGFR treatments, a 4-degree scale system describing the characteristics of the different grades of toxicity (G0 as no toxicity, G1 as mild toxicity, G2 as moderate toxicity and G3 as severe toxicity) relating to the different skin side effects commonly appearing during treatment with these drugs (xerosis and itching, nail changes and papulopustular erythema) (Lacouture ME et al., 2010).

Each patient was evaluated at the beginning of each cycle with panitumumab.

We collected blood vials from 19 patients at different predetermined times of treatment in order to evaluate plasma concentrations of lycopene, β-carotene and malondialdehyde (MDA), a biomarker used to measure the level of oxidative stress in the organism (Matsuo I et al., 1983). Blood samples were taken at about 9am at baseline (Day 0), the day after the first administration of lactolycopene/placebo (Day 1), 3 days after the beginning of treatment with lactolycopene/placebo (Day 3), before the beginning of the second cycle with panitumumab (Day 15), before the beginning of the third cycle with panitumumab (Day 29) and before the beginning of the fifth cycle with panitumumab (Day 71). A blood aliquot was collected, allowed to separate at room temperature, before being centrifuged at 2,000 g for 15 min. The plasma thus obtained was immediately frozen at –80 °C for subsequent assays.

Plasma MDA concentration was determined by high performance liquid chromatography (HPLC) with fluorescent detection, based on 2-thiobarbituric acid (TBA) assay. One molecule of MDA reacts with two molecules of TBA to form a pink-coloured, fluorescent (λ ex, 515 nm; λem, 553 nm) derivative or TBA-MDA condensation under acidic conditions and elevated temperatures for an extended reaction time. Analytical recoveries were 99%, within- and between-run CVs were $<$ 3% and $<$ 9% respectively and the detection limit was 0·01 μmol/l (Malondialdehyde in serum/plasma HPLC Kit, Chromsystems Instruments & Chemicals; Grafelfing/Germany) (Tsikas, 2017; Janero, 1990; Domijan et al., 2015). Plasma lycopene and β-carotene concentrations were determined by an

Table 1
Features of patients, treatment and skin toxicity in the lycopene group.

LYCOPENE GROUP							*Kind of G2/G3 skin side effects*			
RandomizationNumber	*Pharmacokinetic*	*Gender*	*Age*	*Therapy Line*	*Panitumumab Administration (cycles)*	*Skintoxicity G2/G3*	*Papulopustularerythema*	*Nailchanges*	*Xerosis/Itch*	*ToxicityDuration (cycles)*
1_7	yes	M	71	1st - with Folfox	6	no	no	no	no	_
1_9	yes	M	78	3rd - monotherapy	6	no	no	no	no	_
1_11	yes	M	82	1st - monotherapy	4	no	no	no	no	_
1_2	yes	F	58	1st - with Folfox	5	yes	yes	yes	yes	2
3_8	yes	M	82	4th -monotherapy	7	yes	yes	no	yes	5
5_1	yes	F	62	1st with Folfox	4	no	no	no	no	_
5_8	yes	M	74	1st with Folfox	6	no	no	no	no	_
5_10	yes	M	82	1st with Folfox	4	no	no	no	no	_
5_12	yes	M	66	1st with Folfox	5	no	no	no	no	_
2_1	yes	F	24	1st with Folfox	2	no	no	no	no	_
2_8	no	M	82	2nd with Folfiri	5	no	no	no	no	_
6_2	no	F	62	1st with Folfox	4	yes	yes	no	yes	1
6_7	no	M	59	1st with Folfox	6	no	no	no	no	_
					64					8

Abbreviations: Folfiri = leukovorin, 5-fluorouracil, and irinotecan; Folfox = leukovorin, 5-fluorouracil, and oxaliplatin.

Table 2
Features of patients, treatment and skin toxicity in the placebo group.

PLACEBO GROUP							*Kind of G2/G3 skin side effects*			
RandomizationNumber	*Pharmacokinetic*	*Gender*	*Age*	*Therapy Line*	*Panitumumab Administration (cycles)*	*Skintoxicity G2/G3*	*Papulopustularerythema*	*Nailchanges*	*Xerosis/Itch*	*ToxicityDuration (cycles)*
1_1	yes	F	74	1st with Folfox	12	yes	yes	no	no	7
1_8	yes	M	68	2nd with Folfiri	1	yes	yes	no	no	1
1_10	yes	M	86	4th monotherapy	4	no	no	no	no	_
1_12	yes	M	76	1st with Folfox	10	yes	yes	no	no	3
3_7	yes	M	73	1st with Folfox	3	no	no	no	no	_
5_7	yes	M	55	1st with Folfox	5	no	no	no	no	_
5_9	yes	M	81	2nd with Folfox	3	yes	yes	no	no	3
5_11	yes	M	58	1st with Folfox	6	yes	yes	no	yes	6
2_2	yes	F	39	2nd with Folfiiri	5	yes	yes	yes	yes	2
2_7	yes	M	65	2nd with Folfiri	12	yes	yes	no	no	4
6_1	no	F	59	1st with Folfox	6	no	no	no	no	_
6_8	no	M	76	1st with Folfox	6	yes	yes	no	yes	2
6_9	no	M	55	1st with Folfox	6	no	no	no	no	_
7_1	no	F	48	1st with Folfox	6	yes	yes	no	yes	4
9_7	no	M	72	4th with Folfiri	7	yes	yes	yes	yes	4
					92					36

Abbreviations: Folfiri = leukovorin, 5-fluorouracil, and irinotecan; Folfox = leukovorin, 5-fluorouracil, and oxaliplatin.

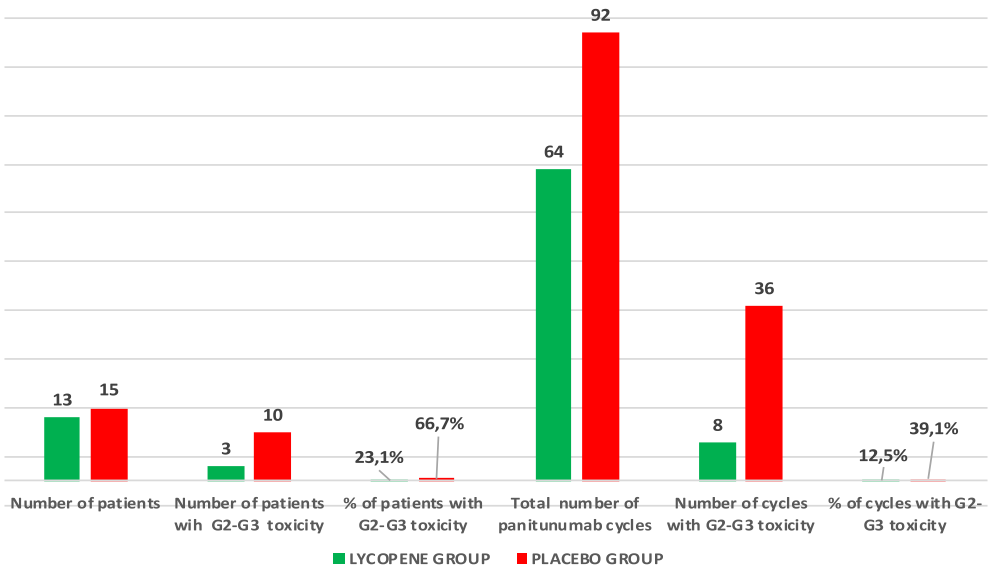

Fig. 1. Difference in ≥ G2 skin toxicity frequency between lactolycopene group (green) and placebo (red) during panitumumab containing treatment. Data are relative to the number of patients and number of panitumumab cycles administered in the two groups.

isocratic HPLC system with UV/VIS detection. The sample clean-up procedure combines a fast precipitation and extraction step. A special RP-chromatography procedure ensures a reliable separation of lycopene and α-, *cis*-β- and all-*trans*-β-carotene as well as some further carotenoids in <10 min. The analyses are quantified by the inclusion of an internal standard, which is a non-naturally occurring carotenoid-derivative, so that only a single detection wavelength is required (453 nm). Analytical recovery was 107%, within- and between-run CVs were < 0·8% and < 2·3% respectively and the detection limit was 5·0 ng/ml/l (ß-Carotene in serum/plasma HPLC Kit, Chromsystems Instruments & Chemicals; Grafelfing/Germany) (Tiwari et al., 2019; Franko et al., 1998; Talwar et al., 1998). All data were collected in an anonymized paper format and then recorded in a secure database in the Oncology Division of the San Carlo Borromeo Hospital in Milan.

All data were collected in an anonymized paper format and then recorded in a secure database in the oncology division of the San carlo borromeo Hospital in Milan.

2.4. Outcomes

The primary endpoint of the PASTO study was to verify the effectiveness of lycopene v. placebo in reducing skin toxicity in patients treated with panitumumab, by analysing differences in: a) mean grade of skin toxicity during the treatment, b) frequency and duration of ≥ G2 skin toxicity, and c) frequency of tetracycline administration. Then, lycopene, β-carotene and MDA plasma concentrations were analysed to test lycopene antioxidant efficacy during panitumumab-containing treatments.

2.5. Statistical analysis

The sample size for the phase-II PASTO clinical trial was calculated to satisfy the primary objective of the study, i.e. clinical effectiveness on skin toxicity, in accordance with the following assumptions: (a) the proportion of patients with grade 2–3 toxicity in the control group is 80% (Van Custen E et al., 2007), (b) an absolute reduction of grade 2–3

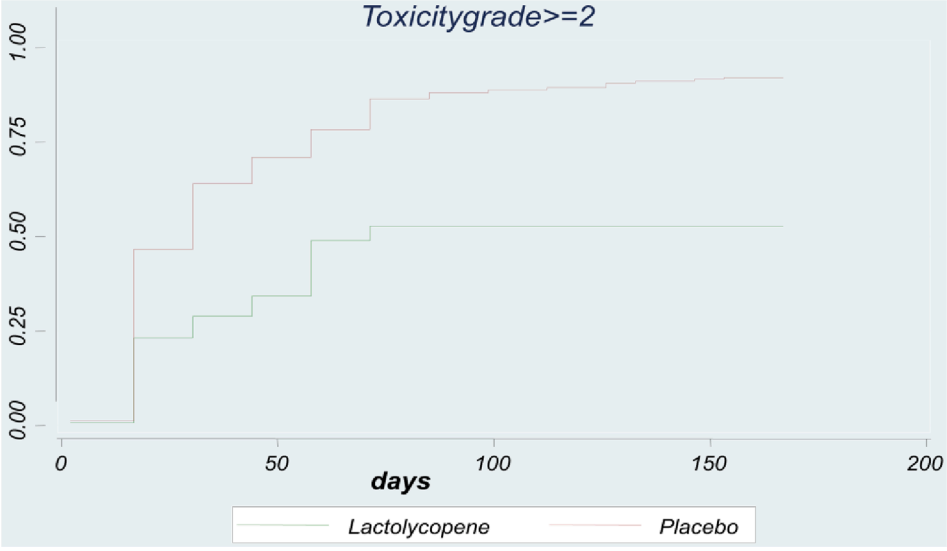

Fig. 2. Time from the initial lactolycopene/placebo treatment to the appearance of a ≥ G2 skin toxicity: difference between the two groups (p = 0.0007 log-rank test).

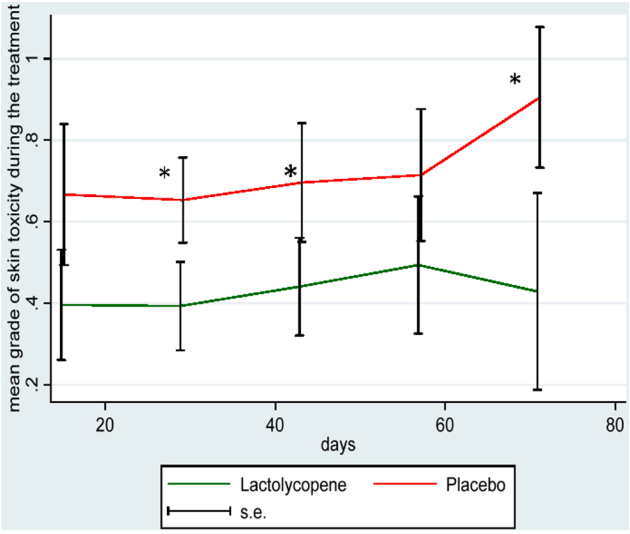

Fig. 3. Mean skin toxicity values at each time point (bars represent standard errors). A $p < 0.05$* was shown at specific time points of the lactolycopene/placebo treatment.

toxicity of 30% in the experimental group, (c) power of 80%, (d) a type I error (with two tails) of 5%, (e) 1:1 ratio in enrolment in both groups, and (f) a Fisher exact test to assess the differences with a 95% confidence interval (CI) – 100 patients would have to be enrolled, calculating a 10% drop-out (90 patients necessary for assessment). Toxicity events were represented with a Kaplan-Meier curve and compared with a log-rank test. Mean toxicity values were represented at each time point (bars represent standard errors [s.e.]). A multivariable mixed effect ordinal logistic regression model is fitted. The analysis program used was v 16.0 (Stata Corp USA).

The double-blind was opened after the first 28 patients showed unusually low skin toxicity compared to that commonly seen in clinical practice during panitumumab-containing treatments. Two enrolled patients with no or low skin toxicity during panitumumab treatment before surgery for resectable liver metastases, showed a moderate-severe toxicity with the resumption of panitumumab after surgery, having come out of the clinical trial. An interim analysis of data was undertaken by the investigators so that study design could be improved and to enlarge recruitment from other institutions. A specific report notified the Ethics Committees before opening the double blind.

The concentrations of lycopene, β-carotene and MDA in the plasma of the two groups were analysed by descriptive statistics for pharmacokinetic parameters.

3. Results

We analysed skin toxicity in 13 patients treated with lactolycopene and 15 patients treated with placebo. Characteristics of patients and skin toxicities are summarized in Tables 1 and 2

Taking G0 + G1 together as representing no-low toxicity and G2 + G3 as moderate-severe toxicity, 10 of the 15 patients in the placebo group (66.7%) developed a ≥ G2 skin toxicity relative to any kind of skin side effect commonly appearing in course of panitumumab treatment compared to 3 of the 13 patients (23.1%) in the lycopene group. In the placebo group, a ≥ G2 skin toxicity occurred during 36 of the cumulative 92 panitumumab cycles (39.1%). In contrast, a ≥ G2 skin toxicity was recorded in only 8 of the 64 cumulative cycles (12.5%) in the lycopene group (Fig. 1). Briefly, an absolute reduction of skin grade 2–3 toxicity in 41% of patients and 46% of panitumumab cumulative cycles was shown in the experimental group versus placebo. The time from the initial lactolycopene/placebo treatment to the appearance of a ≥ G2 skin toxicity was significantly different in the two groups ($p = 0.0007$ log-rank test) (Fig. 2). Panitumumab administration was delayed by one week due to skin toxicity in 4 of the 15 patients (26.7%) in 6 of the 92 cumulative cycles (6.5%) in the placebo group. No delay in the scheduled treatment was needed in the lycopene group. A $p\ value < 0.05$ for the mean value of skin toxicity was shown at specific times of the treatment (Fig. 3) despite the increased use of tetracyclines in the placebo group. A multivariate analysis was performed to evaluate factors possibly influencing the difference in skin toxicity in the two groups (Table 3).

According to the protocol, tetracyclines were administered for ≥ G2 skin toxicity in 3 of the 13 patients (23.1%) during 8 of the 64 cumulative cycles (12.5%) in the lycopene group and 10 of the 15 patients (66.7%) during 36 of the 92 cumulative cycles (39.1%) in the placebo group.

Lycopene, β-carotene and MDA concentrations were evaluated in the plasma of 10 patients treated with lactolycopene and 9 patients treated with placebo at different times of treatment. Lycopene, β-carotene and MDA concentrations were analysed as the difference between the mean value of each component during lactolycopene/placebo treatment (Day1 + Day 3 + Day 15 + Day29 + Day 71) and the value of the same component at Day 0 compared to the value of the same component at Day 0 (lycopene index, β-carotene index and MDA index, respectively) (Table 4).

Lycopene was depleted during panitumumab treatment, with a mean lycopene index decrease of –141.9% in the placebo group, and with 5 out of 9 patients showing a lower mean plasma concentration during antineoplastic treatment than at Day 0; the β-carotene index also decreased –157.2% in the placebo group, with 8 out of 9 patients showing a lower mean plasma concentration during treatment than at Day 0.

When patients were supported with lactolycopene, the mean lycopene index increased ± 94.2%, with 6 out of 10 patients showing a higher mean concentration during antineoplastic treatment compared to Day 0. In the same group, mean β-carotene index decreased –105.5%, although 4 out of 10 patients had an increased mean plasma concentration during antineoplastic treatment compared to Day 0 (Fig. 4).

The mean MDA index was extremely high in the placebo group, with

Table 3
Multivariate analysis of factors possibly influencing the difference in skin toxicity in the two groups.

c	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
Age	-.0022646	.0540356	-0.04	0.967	-.1081724	.1036433
_IGender_2	-.4648677	1.57349	-0.30	0.768	-3.548852	2.619116
Lineoftherapy	.1076025	.6235597	0.17	0.863	-1.114552	1.329757
_IGroup_2	-2.578265	1.209811	-2.13	0.033	-4.949451	-.2070795
tetrac	-.1319483	.5531717	-0.24	0.811	-1.216145	.9522484

Multivariate analysis.
Abbreviations: Gender 2: male; Group 2: Treated with laclolycopene; Tetrac: Use of tetracyclines; coef.: coefficient; z: standardized normal distribution; p: p-value.

Table 4
Lycopene, β-carotene and MDA concentrations in the plasma of each patient at baseline, different times during panitumumab containing therapy and as mean value of each component in patients treated with lactolycopene (green) and patients treated with placebo (red). Lycopene, β-carotene and MDA Indexes are calculated as the difference between the mean concentration of each component during lactolycopene/placebo treatment (Day 1 + Day 3 + Day 15 + Day 29 + Day71) and the value of the same component at Day 0 compared to the value of the same component at Day 0, for each patient.

SUBJECT RANDOMIZATION NUMBER		1_1	1_2	1_7	1_8	1_9	1_10	1_11	1_12	3_7	3_8	5_7	2_1	2_7	5_1	5_8	5_9	5_10	5_11	5_12
Day 0	LYCOPENE ng/ml	1026	377	753	300	1071	464	531	537	140	493	70	178	883	199	420	162	548	388	543
	β-CAROTENE ng/ml	297	130	703	219	437	293	32	275	95	193	140	390	646	177	47	129	256	57	148
	MDA µM	0,55	0,57	0,52	0,37	0,44	0,5	0,5	0,9	0,11	1,11	0,6	0,85	1,08	0,18	0,18	0,22	0,16	0,13	0,39
Day 1	LYCOPENE ng/ml	1039	402	702	278	1245	292		487	144	407	76	362	637	251	463	306	559	340	646
	β-CAROTENE ng/ml	267	140	634	174	407	285		257	117	247	129	406	545	184	76	137	245	50	152
	MDA µM	0,58	0,56	0,65	0,62	0,62	0,46		0,79	0,63	0,43	0,66	0,82	0,93	0,19	0,18	0,13	0,27	0,14	0,12
Day 3	LYCOPENE ng/ml	1159	413	834	313	1435		583	551	121	497	69	131	910	355	341	228	408	443	632
	β-CAROTENE ng/ml	278	150	642	197	433		40	275	106	255	143	320	620	216	56	119	176	62	139
	MDA µM	0,56	0,66	0,36	0,54	0,92		0,42	0,62	0,65	0,46	0,75	1,27	1,28	0,16	0,19	0,11	0,21	0,18	0,1
Day 15	LYCOPENE ng/ml	533	793	686	302	1136	589	583	428	65	645	45	297	628	156	361	151	164	337	461
	β-CAROTENE ng/ml	212	185	405	173	343	266	39	249	66	268	115	426	328	122	83	127	98	52	138
	MDA µM	0,66	0,52	0,36	0,58	0,69		0,51	0,72	0,68	0,6		0,98	1	0,15	0,15	0,15	0,15	0,28	0,13
Day 29	LYCOPENE ng/ml	517	695	682	177	1146	712	519	411	78	590	32		914	574	243	117	207	384	240
	β-CAROTENE ng/ml	264	199	336	163	401	215	28	173	82	376	83		483	166	41	108	108	58	63
	MDA µM	0,52	0,62	0,43	0,49	0,66	0,56	0,47	0,49	0,82	0,41			0,88	0,31	0,31	0,12	0,1	0,35	0,16
Day 71	LYCOPENE ng/ml	613		644		898					547	54		1472	205	140			545	
	β-CAROTENE ng/ml	299		347		222					360	74		471	132	49			93	
	MDA µM	0,57		0,41		0,75					0,4	0,58		0,96	0,22	0,23			0,19	
Mean Value	LYCOPENE ng/ml	772,2	575,8	709,6	267,5	1172	531	561,7	469,3	102	537,2	55,2	263,3	912,2	308,2	309,6	200,5	334,5	409,8	494,8
	β-CAROTENE ng/ml	264	168,5	472,8	176,75	361,2	255,33	35,67	238,5	92,75	301,2	108,8	384	489,4	164	61	122,75	156,75	63	123
	MDA µM	0,58	0,59	0,44	0,56	0,73	0,51	0,47	0,66	0,7	0,46	0,66	1,02	1,01	0,21	0,21	0,13	0,18	0,23	0,13
Index	LYCOPENE %	-24,70%	52,70%	-5,80%	-10,80%	9,40%	14,40%	5,80%	-12,60%	-27,10%	9,00%	-21,10%	47,90%	3,30%	54,90%	-26,30%	23,80%	-39,00%	5,60%	-8,90%
	β-CAROTENE %	-11,10%	29,60%	-32,70%	-19,30%	-17,30%	-12,90%	11,50%	-13,30%	-2,40%	56,10%	-22,30%	-1,50%	-24,2,%	-7,30%	29,80%	-4,80%	-38,80%	10,50%	-16,90%
	MDA %	2,20%	3,50%	-15,00%	50,70%	65,50%	2,00%	-6,70%	-27.2%	531,80%	-58,60%	10,60%	20,40%	-6,50%	14,40%	17,80%	-42,00%	14,10%	75,40%	-67,30%

Abbreviation: MDA = malondialdehyde.

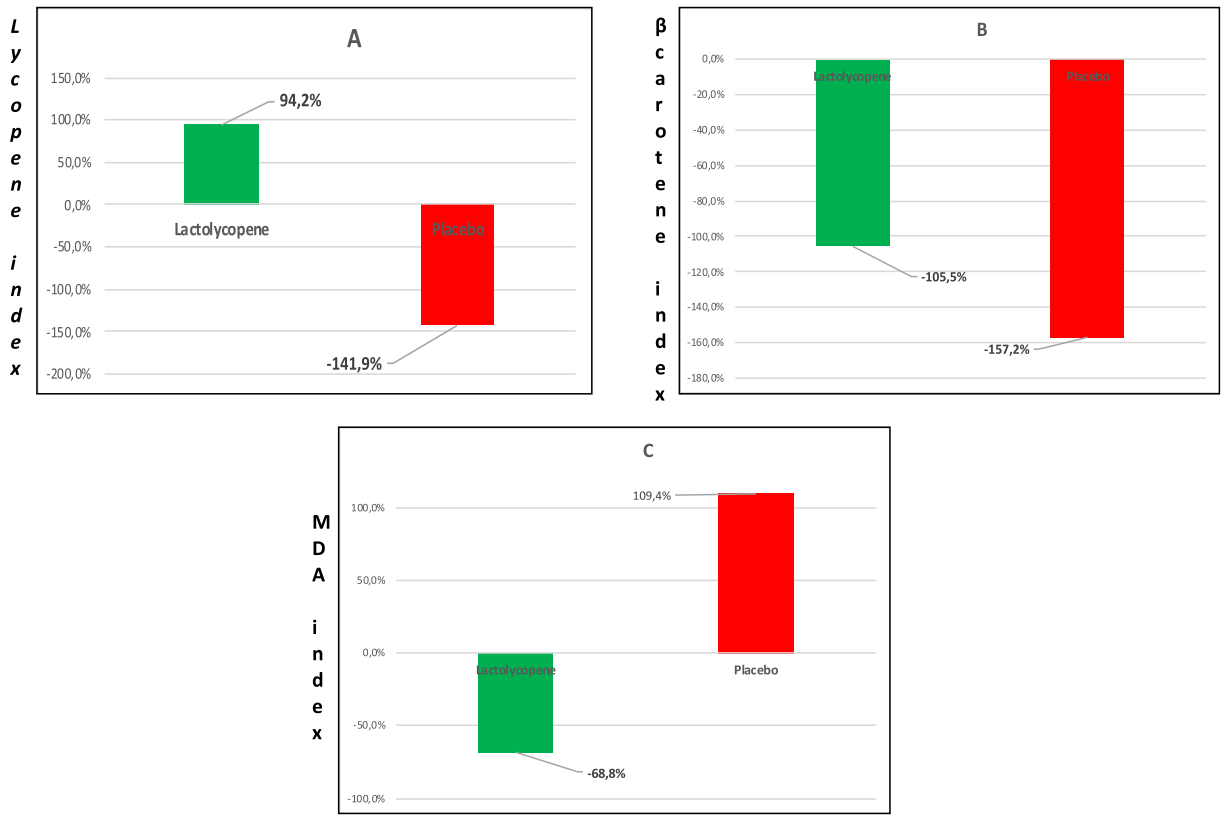

Fig. 4. Mean lycopene index (**4a**), mean β-carotene index (**4b**) and mean MDA index (**4c**): difference between lactolycopene group (green) and placebo (red). Mean Indexes are obtained through the mean of lycopene, β-carotene and MDA indexes of each patient calculated as the difference between the mean compound concentration in the plasma during treatment (Day1 + Day 3 + Day 15 + Day 29 + Day 71) and the value of the same compound at Day 0, compared to the value of the same compound at Day 0, for each patient.

an increased value of + 109.4%, and 6 out of 9 patients showing a higher mean concentration during treatment compared to Day 0. In patients supported with lactolycopene, the mean MDA index decreased –68.8%, with 4 out of 10 patients showing a lower mean concentration during treatment than at Day 0 (Fig. 4).

4. Discussion

Regulation of EGFR signalling plays a major role in protecting cells against oxidative stress and inhibition of the EGFR pathway induces cell injury and killing due to oxidative stress (Chen et al., 2007; Wang et al., 2000; Orcutt et al., 2011). Lycopene is a compound with a considerable antioxidant action. Prolonged use in the diet of β-carotene in general, and lycopene in particular, is effective in protecting skin from ageing, and sunlight and radiotherapy damage (Aghajanpour M et al., 2017; Grether-Beck S et al., 2017; Petyaev IM et al., 2018).

We obtained similar reductions in skin toxicity to those described for tetracycline systemic administration using lactolycopene in panitumumab-treated patients. Tetracyclines have been shown to be effective in reducing skin toxicity following treatment with EGFR inhibitors (Hofheinz RD et al., 2016; Jatoi A et al., 2008; Scope A et al., 2007), mostly through their anti-inflammatory efficacy in reducing neutrophil chemotaxis and epidermal keratinocyte and macrophage activation (Webster G et al., 2007). Recently, the results of randomized clinical trials showed significant efficacy of prophylactic use of tetracyclines in decreasing skin toxicity (Arrieta O et al., 2015; Melosky B et al., 2016). Although tetracyclines are a class of antibiotics with a low toxic profile, they can cause side effects such as photosensitivity, dizziness, asthenia, liver toxicity and stomach or bowel upsets. Moreover, in the presence of a pre-existing renal disease, tetracyclines can accumulate in the organism causing hyperazotemia and metabolic acidosis (Smilack JD, 1999), especially during prolonged administration of the drug. These side effects can be problematic in patients with metastatic cancer undergoing oncologic treatment.

In our series, the more frequent administration of tetracyclines in the placebo compared to the lycopene group was due to the more frequent ≥ G2 skin toxicity in the former cohort and could even have underestimated the real effectiveness of lycopene in decreasing skin toxicity. No toxic effect due to lycopene was observed in our series, and no toxic effect has been attributed to lycopene in the literature. We chose not to submit patients to a dietary regime, restricted in lycopene and other antioxidant nutrients, to avoid dietary restrictions for patients with frequently observed nutritional problems and to check the real impact of lactolycopene administration under the usual nutritional conditions for this population. Other factors which may affect our results include: different capacities of absorption and metabolism of lycopene, the contemporaneous presence or absence of oxidative events other than antineoplastic treatment in each patient and, as described above, the unbalanced use of tetracyclines in the two groups.

Analysis of plasma samples obtained at different times during panitumumab-containing therapy showed that lycopene was depleted during antineoplastic treatment in the placebo group and similar results were obtained for β-carotene. The mean concentration of lycopene increased during antineoplastic treatment with lactolycopene support, with a smaller decrease in mean β-carotene concentration than that seen in the placebo group. This suggests that lactolycopene administration may replenish the exhausted lycopene and could partially save other antioxidants from the consumption caused by oxidative stress during panitumumab treatment.

MDA is a compound arising from peroxidation of polyunsaturated fatty acids. ROS degrade polyunsaturated lipids forming MDA and this compound is one of the main reactive electrophile species that cause toxic stress in cells by forming covalent protein adducts referred to as

advanced lipid oxidation end-products. The degree of lipid peroxidation can be estimated from the amount of MDA and, for this reason, the concentration of this compound is specifically tested to evaluate skin toxicity during oxidative stress (Matsuo I et al., 1983). Mean MDA concentration rose during treatment in the placebo group, whereas lactolycopene administration protected tissues from oxidative stress since the mean MDA concentration during antineoplastic therapy was lower than the MDA concentration at Day 0.

5. Conclusions

Despite the concerns described above and the limited number of patients, our results clearly show the effectiveness of lycopene in reducing skin toxicity and tetracycline administration and protecting tissues from oxidative stress in patients treated with panitumumab. In our opinion, considering the absence of any side effect of this therapy, except for intolerance or allergy to tomato or milk, lactolycopene, which has to be considered a nutraceutical compound rather than a drug, could already be used in clinical practice while waiting for a larger study able to verify its real impact in clinical practice and how to better integrate its use with tetracycline administration so that skin toxicity in patients treated with anti-EGFR therapies can be minimized.

CRediT authorship contribution statement

Mauro Moroni: Conceptualization, Methodology, Validation, Writing - original draft, Supervision, Project administration. **Marco Pirovano:** Conceptualization, Methodology, Validation, Software, Visualization. **Silvia Brugnatelli:** Conceptualization, Methodology, Resources. **Martina Zucca:** Data curation, Validation, Visualization. **Marco Morreale:** Data curation, Visualization. **Vittoria Rizzo:** Methodology, Investigation, Resources. **Alessandra Ferrari:** Methodology, Data curation. **Carmine Tinelli:** Formal analysis, Software. **Annalisa De Silvestri:** Formal analysis, Validation. **Maurizio Meregalli:** Resources, Validation. **Monica Giordano:** Methodology, Resources. **Salvatore Artale:** Methodology, Resources. **Massimiliano Cergnul:** Resources. **Roberto Bollina:** Resources. **Mimma Rizzo:** Resources. **Paolo Pedrazzoli:** Conceptualization, Methodology, Validation, Writing - original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The funders had no role in the writing of the report and the study design, data collection, data analysis or data interpretation and have no rights on data property. The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions or policies of the institutions with which they are affiliated.

Disclosure

MM1 is the vice-president and MP the treasurer of AMOlavitaONLUS. AMOlavitaONLUS is the sole owner and it holds all the ownership rights to the international patent application entitled "Use of carotenoid derivatives to reduce the toxicity and increase the efficacy of anti-EGFR antitumour treatments" (N°PCT/IB2018/050229). MM1 and MP are exclusively inventors of the patent and they have no ownership rights to the patent. Indena S.p.A., which supplied lactolycopene free of charge for the Pasto Clinical Trial, is not the owner of the patent for lactolycopene production.

Ethics statement

This article is solely the work of the authors stated, it hasn't been previously published elsewhere and it is not under consideration by another journal. Results of this clinical trial were previously posted, as a presentation preprint, in "Preprints with the Lancet". Its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. The study has been carried out in accordance with "The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript is in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. If accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright holder.

All Authors have given their approval of the version to be submitted after revising it critically.

References

Aghajanpour, M., Nazer, M. R., Obeidavi, Z., Akbari, M., Ezati, P., & Kor, N. M. (2017). Functional foods and their role in cancer prevention and health promotion: A comprehensive review. *Am. J. Cancer. Res., 7*, 740–769. PMID: 28469951.

Arrieta, O., Vega-Gonzales, M. T., López-Macías, D., Martinez-Hernández, J. N., Bacon-Fonseca, L., Macedo-Pérez, E. O., … De La Garza, J. (2015). Randomized, open-label trial evaluating the preventive effect of tetracycline on afatinib induced-skin toxicities in non-small cell lung cancer patients. *Lung Cancer., 88*, 282–288. https://doi.org/10.1016/j.lungcan.2015.03.019.

Balić, A., & Mokos, M. (2019). Do we utilize our knowledge of the skin protective effects of carotenoids enough? *Antioxidants (Basel)., 8*, 259. https://doi.org/10.3390/antiox8080259.

Ben-Dor, A., Steiner, M., Gheber, L., Danilenko, M., Dubi, N., Linnewiel, K., … Levy, J. (2005). Carotenoids activate the antioxidant response element transcription system. *Molecular Cancer Therapeutics, 4*, 177–186. PMID: 15657364.

Busam, K. J., Capodieci, P., Motzer, R., Kiehn, T., Phelan, D., & Halpern, A. C. (2001). Cutaneous side-effects in cancer patients treated with the antiepidermal growth factor receptor antibody C225. *British Journal of Dermatology, 144*, 1169–1176. https://doi.org/10.1046/j.1365-2133.2001.04226.x.

Chen, Y., Jungsuwadee, P., Vore, M., Butterfield, D. A., & St Clair, D. K. (2007). Collateral damage in cancer chemotherapy: Oxidative stress in non-target tissues. *Mol. Interv., 7*, 147–156.https://doi.org/10.1124/mi.7.3.6.

Conner, E. M., & Grisham, M. B. (1996). Inflammation, free radicals and antioxidants. *Nutrition., 12*, 274–277. https://doi.org/10.1016/S0899-9007(96)00000-8.

Di Mascio, P., Kaiser, S., & Sies, H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics, 274*, 532–538. https://doi.org/10.1016/0003-9861(89)90467-0.

Domijan, A. M., Ralić, J., RadićBrkanac, S., Rumora, L., & Žanić-Grubišić, T. (2015). Quantification of malondialdehyde by HPLC-FL - application to various biological samples. *Biomedical Chromatography, 29*, 41–46. https://doi.org/10.1002/bmc.3361.

Franko, M., Van de Bovenkamp, P., & Bicanic, D. (1998). Determination of trans-beta-carotene and other carotenoids in blood plasma using high-performance liquid

chromatography and thermal lens detection. *J. Chromatog. B. Biomed. Sci. Appl., 718*, 47–54. https://doi.org/10.1016/S0378-4347(98)00347-8.

Grether-Beck, S., Marini, A., Jaenicke, T., Stahl, W., & Krutmann, J. (2017). Molecular evidence that oral supplementation with lycopene or lutein protects human skin against ultraviolet radiation: Results from a double-blinded, placebo-controlled, crossover study. *British Journal of Dermatology, 176*, 1231–1240. https://doi.org/10.1111/bjd.15080.

Hofheinz, R. D., Deplanque, G., Komatsu, Y., Kobayashi, Y., Ocvirk, J., Racca, P., … Jatoi, A. (2016). Recommendations for the prophylactic management of skin reactions induced by epidermal growth factor receptor inhibitors in patients with solid tumors. *The Oncologist, 21*, 1483–1491. https://doi.org/10.1634/theoncologist.2016-0051.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic. Biol. Med., 9*, 515–540. https://doi.org/10.1016/0891-5849(90)90131-2.

Jatoi, A., Rowland, K., Sloan, J. A., Gross, H. M., Fishkin, P. A., Kahanic, S. P., … Loprinzi, C. L. (2008). Tetracyclines to prevent epidermal growth factor receptor inhibitor-induced skin rashes: Results of a placebo-controlled trial from the North Central Cancer Treatment Group (N03CB). *Cancer, 113*, 847–853. https://doi.org/10.1002/cncr.23621.

Lacouture, M. E., Maitland, M. L., Segaert, S., Setser, A., Baran, R., Fox, L. P., … Trotti, A. (2010). A proposed EGFR inhibitor dermatologic adverse event-specific grading scale from the MASCC skin toxicity study group. *Supportive Care in Cancer, 18*, 509–522. https://doi.org/10.1007/s00520-009-0744-x.

Matsuo, I., Yoshino, K., & Ohkido, M. (1983). Mechanism of skin surface lipid peroxidation. *Current Problems in Dermatology, 11*, 135–143. https://doi.org/10.1159/000408670.

Melosky, B., Anderson, H., Burkes, R. L., Chu, Q., Hao, D., Ho, V., … Laskin, J. J. (2016). Pan Canadian Rash Trial: A randomized phase III trial evaluating the impact of a prophylactic skin treatment regimen on epidermal growth factor receptor- tyrosine kinase inhibitor-induced skin toxicities in patients with metastatic lung cancer. *Journal of Clinical Oncology, 34*, 810–815. https://doi.org/10.1200/JCO.2015.62.3918.

Orcutt, K. P., Parsons, A. D., Sibenaller, Z. A., Scarbrough, P. M., Zhu, Y., Sobhakumari, A., … Simons, A. L. (2011). Erlotinib-mediated inhibition of EGFR signaling induces metabolic oxidative stress through NOX_4. *Cancer Research, 71*, 3932–3940. https://doi.org/10.1158/0008-5472.CAN-10-3425.

Petyaev, I. M., Zigangirova, N. A., Pristensky, D., Chernyshova, M., Tsibezov, V. V., Chalyk, N. E., … Bashmakov, Y. (2018). Non-invasive immunofluorescence assessment of lycopene supplementation status in skin smears. *Monoclon. Antib. Immunodiagn. Immunother., 37*, 139–146. https://doi.org/10.1089/mab.2018.0012.

Richelle, M., Bortlik, K., Liardet, S., Hager, C., Lambelet, P., Baur, M., Applegate, L.A., Offord., E.A., 2002. A food-base formulation provides lycopene with the same bioavailability to humans as that from tomato paste. J. Nutr. 132, 404-408. https://doi.org/10.1093/jn/132.3.404.

Scope, A., Agero, A. L. C., Dusza, S. W., Myskowski, P. L., Lieb, J. A., Saltz, L., … Halpern, A. C. (2007). Randomized double-blind trial of prophylactic oral minocycline and topical tazarotene for cetuximab-associated acne-like eruption. *Journal of Clinical Oncology, 25*, 5390–5396. https://doi.org/10.1200/JCO.2007.12.6987.

Smilack, J. D. (1999). *The Tetracyclines. Mayo Clinic. Proc., 74*, 727–729. https://doi.org/10.4065/74.7.727.

Talwar, D., Ha, T. K., Cooney, J., Brownlee, C., & O'Reilly, D. S. (1998). A routine method for the simultaneous measurement of retinol, alpha-tocopherol and five carotenoids in human plasma by reverse phase HPLC. *Clinica Chimica Acta, 270*, 85–100. https://doi.org/10.1016/S0009-8981(97)00224-6.

Tiwari, S., Upadhyay, N., Singh, A. K., Meena, G. S., & Arora, S. (2019). Organic solvent free extraction of carotenoids from carrot bio-waste and its physico-chemical properties. *Journal of Food Science and Technology, 56*, 4678–4687. https://doi.org/10.1007/s13197-019-03920-5.

Tsikas, D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *AnnalBiochem., 524*, 13–30. https://doi.org/10.1016/j.ab.2016.10.021.

Van Cutsem, E., Peeters, M., Siena, S., Humblet, Y., Hendlisz, A., Neyns, B., … Amado, R. G. (2007). Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *Journal of Clinical Oncology, 25*, 1658–1664. https://doi.org/10.1200/JCO.2006.08.1620.

Wang, X., McCullough, K. D., Franke, T. F., & Holbrook, N. J. (2000). Epidermal growth factor receptor dependent Akt activation by oxidative stress enhances cell survival. *Journal of Biological Chemistry, 275*, 14624–14631. https://doi.org/10.1074/jbc.275.19.14624.

Webster, G., & Del Rosso, J. Q. (2007). Anti-inflammatory activity of tetracyclines. *Dermatologic Clinics, 25*, 133–135. https://doi.org/10.1016/j.det.2007.01.012.

Weijl, N. I., Cleton, F. J., & Osanto, S. (1997). Free radicals and antioxidants in chemotherapy-induced toxicity. *Cancer Treatment Reviews, 23*, 209–240. https://doi.org/10.1016/s0305-7372(97)90012-8.

Young, A. J., & Lowe, G. M. (2001). Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics, 385*, 20–27. https://doi.org/10.1006/abbi.2000.2149.