Clinical Study Protocol

A randomised, double-blind, vehicle-controlled, multi-centre, parallel-group study to investigate the safety, tolerability, and efficacy of GR1014 cutaneous gel as a topical radioprotector in the prevention of the radiodermatitis occurring with adjuvant radiotherapy for localised breast cancer after lumpectomy

GuARD: GR1014 cutaneous gel Against Radiation Dermatitis

Protocol number:	GRA.05.SPR.0001
Protocol version:	Version 2.0
Status:	Final
Date:	02 Apr 2024
Phase:	Phase 2
Investigational product:	GR1014 Cutaneous Gel
EU CT Number:	2023-508728-36-00
IRAS Number:	1009515
Applicant or Holder/ Sponsor Name:	Graegis Pharmaceuticals Ltd c/o The Cambridge Partnership The Dorothy Hodgkin Building Babraham Research Campus Cambridge CB22 3FH United Kingdom

INVESTIGATOR'S SIGNATURE

I hereby declare that I have read and understood this protocol, all protocol-related documents, and the investigational product's documents.

I agree to conduct the study as outlined and in agreement with the currently applicable international guidelines and the local legal requirements.

Signature of Investigator	**Date** *(DD-MMM-YYYY)*

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DOCUMENT HISTORY

Document	Version Date	Description of changes
Protocol Version 1.0	16 Jan 2024	Initial protocol
Protocol Version 2.0	02 Apr 2024	MHRA & ANSM request for changes

Amended protocol

As necessary, an updated version of this document has been prepared to incorporate all revisions to date, including amendments made at the request of the country's competent authorities, institutional review boards/ethics committees (IRBs/ECs), etc.

Please refer to Appendix 2.

1 PROTOCOL SYNOPSIS

Applicant/Sponsor	Graegis Pharmaceuticals Ltd
Protocol Number / Study Code	GRA.05.SPR.0001 / **GuARD**
Protocol version/date	Version 2.0 dated 02-Apr-2024
Title of study	A randomised, double-blind, vehicle-controlled, multi-centre, parallel-group study to investigate the safety, tolerability, and efficacy of GR1014 cutaneous gel as a topical radioprotector in the prevention of the radiodermatitis occurring with adjuvant radiotherapy for localised breast cancer after lumpectomy
Short title	GuARD: GR1014 Cutaneous Gel Against Radiation Dermatitis
Document status	Final
Clinical phase	Phase 2
Investigational Product	GR1014 Cutaneous Gel (GR1014-CG)
Dose, method of administration, and duration of treatment:	GR1014-CG 4.7% and 2.4%, a topical formulation of 2-[(3-aminopropyl) amino] ethanethiol (amifostine thiol, WR-1065, GR1014), the active metabolite of amifostine (Ethyol®). Vehicle gel, the same topical formulation without amifostine thiol. GR1014-CG or vehicle gels are to be applied topically on the skin surface to be irradiated (0.5 mL per 100 cm^2). **IMP administration**: Approximately 15 to 30 min before each radiation therapy (RT) session, the investigational medicinal product (IMP) is to be applied generously by the participant over the whole zone to be irradiated with a gentle massage until complete gel formation, using disposable nitrile-gloved hands, under a health care provider's supervision. The product is to be left on the skin for 15 min. The treated skin is to be thereafter gently cleansed and carefully patted dry before participant RT.

	IMP Handling The IMP is to be stored in a freezer and thawed 30 min to 1 hour prior to its application by the participant. Alternatively, it can be taken out of the freezer in the morning and stored in a refrigerator until 30 min before application. A vial should not be left at room temperature for more than 4 hours.
Planned study duration	12 months from the first patient - first visit to the last patient - last visit.
Rationale	The involvement of reactive oxygen species in the development of radiodermatitis suggests that reducing their production or scavenging them from tissue could be a potential strategy for prevention. Aminothiols, including amifostine, are well-established radioprotective agents. In RT, amifostine (a prodrug acting via its active metabolite, amifostine thiol) is approved to reduce dry mouth (xerostomia) in patients undergoing post-operative radiation treatment for head and neck cancer, especially when the parotid glands are irradiated. However, using amifostine as a radioprotector for breast cancer patients faces significant barriers due to impractical intravenous (IV) administration for outpatients and associated side effects like severe orthostatic hypotension, rash, nausea, and vomiting. To overcome these challenges, a topical gel containing the active metabolite amifostine thiol (GR1014), called GR1014-CG, was designed to be applied directly to the skin before each RT session, delivering a sufficient concentration of amifostine thiol to the epidermis, thereby achieving its radioprotective effect while limiting systemic exposure and reducing the risk and seriousness of systemic adverse events associated with amifostine treatment. This study aims to examine the safety profile of GR1014-CG in patients undergoing RT and its ability to reduce radiation-induced dermatitis.
Objectives	Primary Objective: • To investigate the safety, tolerability, and efficacy of GR1014-CG (4.7%; 2.4%) as a treatment to prevent radiodermatitis occurring with adjuvant ultra

	hypofractionated RT for localised, non-metastatic breast cancer after lumpectomy, versus vehicle gel. <u>Secondary Objectives:</u> • To assess the efficacy of GR1014-CG (4.7%; 2.4%) - in reducing radiodermatitis severity versus vehicle gel. - in reducing peak radiodermatitis severity versus vehicle gel. - in reducing radiodermatitis emergence versus vehicle gel. - in delaying the onset of radiodermatitis versus vehicle gel. - in reducing the duration of radiodermatitis versus vehicle gel. - in reducing severe radiodermatitis versus vehicle gel. - in reducing the side effects of RT other than dermatitis versus vehicle gel. - in reducing patient-reported pain intensity in the irradiated skin region induced by RT versus vehicle gel. - in reducing patient-reported pruritus intensity in the irradiated skin region induced by RT versus vehicle gel. - on participants' assessment of their quality of life during and after RT versus vehicle gel. • To characterise the plasma C_{max} of amifostine thiol after application of GR1014-CG. <u>Exploratory Objectives:</u> • To assess the efficacy of GR1014-CG in reducing the skin hyperaemia during and after RT versus vehicle gel. • To assess the ability of GR1014-CG in preventing the loss of integrity of the skin barrier function during and after RT versus vehicle gel.
Endpoints	<u>Primary Efficacy Endpoint:</u> Percentage of participants with no radiation dermatitis (Common Terminology Criteria for Adverse Events latest version [CTCAE LV] grade of 0) between the first RT session and 4 weeks after the last one. <u>Secondary Efficacy Endpoints:</u> 1. Percentage of participants with a radiodermatitis grade ≥2 (CTCAE LV) occurring at any time between the first RT session and 4 weeks after the last one – key secondary.

	2. Percentage of participants with no radiation dermatitis (CTCAE LV, grade of 0), 4 weeks after the last RT session (at Visit 10). 3. Maximum grade of radiodermatitis (CTCAE LV) reached in each participant between the first RT session and 4 weeks after the last one. 4. Assessments of radiodermatitis grade (CTCAE LV) between the first RT session and the last visit. 5. Time to onset of radiodermatitis grade ≥2 (CTCAE LV). 6. Duration of radiodermatitis grade ≥2 (CTCAE LV). 7. Percentage of participants with severe radiodermatitis (grade ≥3; CTCAE LV) occurring at any time between the first RT session and 4 weeks after the last one. 8. Number of cases of side effects of RT other than radiodermatitis occurring at any time between the first RT session and the last visit (oedema; hyperpigmentation; skin infection; need for topical and systemic antibiotics; need for topical steroids; need for analgesics; need for silicone-based dressings). *Participant Reported Outcome Assessments* 9. Absolute change from baseline in weekly averaged worst skin pain score in the irradiated area. Worst pain intensity (during the last 24 hours) will be scored daily on a 0-10 Numeric Rating Scale (NRS) from first to last visit. 10. Absolute change from baseline in weekly averaged peak pruritus score in the irradiated area. Peak pruritus intensity (during the last 24 hours) will be scored daily on a 0-10 NRS from first to last visit. 11. Total score and sub-scores of Dermatology Life Quality Index (DLQI) addressed from the first RT session to the last visit. Exploratory Endpoints (at one designated investigational site only): 12. Breast skin surface inflammation evolution will be assessed through a repeated measures analysis of the skin colour a-star (a*) values calculated using a tristimulus colorimetric instrument in accordance with the CIELAB colour systems from the first RT session to the last visit.

	13. Transepidermal water loss (TEWL) measured from the first RT session to the last visit for 4 test areas within the radiation field of the breast (1 site per quadrant) and on a control area of the volar aspect of the forearm on the non-irradiated side. The last visit may be at 4 weeks, 6 weeks or 8 weeks after the last RT session, depending on subject's attainment of radiodermatitis Grade 0 (CTCAE LV). <u>Safety Endpoints:</u> 14. Serious Adverse Reactions (SARs). 15. Adverse Events of Special Interest (AESIs). 16. Percentage of study withdrawal. 17. Stop/delay in RT sessions due to safety events. <u>Pharmacokinetic Endpoints:</u> 18. C_{max} 19. Time to C_{max} (T_{max}) 20. Area under the plasma concentration-time curve from time 0 to the last measurable time point t_{last} (AUC_{last})
Countries/sites	Approximately 10 sites in 3 countries (France, United Kingdom, Poland)
Study population	<u>Inclusion Criteria</u> 1. Dated, signed informed consent obtained from individuals who agree to participate in the study. 2. Female patients with age ≥18 years. Those of childbearing potential [1] must be using highly effective contraception methods during the study and for 30 days after the last administration of the study treatment and have a negative pregnancy test at screening and no more than 10 days prior to the administration of the first dose of study treatment.

[1] A woman is considered of childbearing potential (WOCBP), i.e., fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

	3. Patients with primary, localised breast cancer without metastases pT1--3, pN0--N1mi, M0, who have undergone breast-conserving surgical excision and require adjuvant RT. If adjuvant chemotherapy is delivered, the patient must be randomised within 4 weeks after the last series of adjuvant chemotherapy. If no adjuvant chemotherapy is delivered, the patient must be randomised within 8 weeks from the last surgery. The patient can be included no matter the status of oestrogen and progesterone receptors, malignancy grade, or HER2 status. 4. Eastern Cooperative Oncology Group (ECOG) performance status 0-2. 5. Patients to be treated with ultra hypofractionated RT, 26 Gy in 5 fractions (5.2 Gy) on whole breast (EQD_2 > 42.6 Gy for α/β of 3). 6. Patients with no signs of dermatitis in the breast area to be irradiated, i.e., assessed Grade 0 as per CTCAE LV radiation dermatitis grading. 7. Patients whom the investigator has deemed able to comply with the RT and investigational treatment done under the supervision of the medical personnel throughout the study period. 8. Patients affiliated with the Social Security System (France). 9. Patients who have completed the appropriate washout period for any prior interventions or treatments. <u>Exclusion Criteria</u> 1. Pregnant and breastfeeding women. 2. Patients under any treatment concomitant to RT tested in another clinical study. 3. Allergies to any of the ingredients in GR1014-CG. 4. Patients protected by law (legal guardianship or protection). 5. Patients unable to adhere to the requirements of the study. 6. History of thoracic RT. 7. Participants with the presence of skin rash, ulceration, unhealed surgical wounds, biopsy sites, or open wounds in the breast or chest area at visit 2. 8. Patients suffering from scleroderma, auto-immune disease, micro-vascular diseases, collagen tissue diseases, lupus, pre-existing loss of skin integrity, active eczema in the region to be treated or with a history of any of the following: drug-induced severe cutaneous adverse reaction (SCAR;

	including, but not limited to Stevens-Johnson syndrome/toxic epidermal necrolysis [SJS/TEN], or drug reaction with eosinophilia and systemic symptoms).
Methodology	This is an interventional, vehicle-controlled, multi-centre, double-blinded, parallel-group trial to assess the ability of CR1014-CG to prevent or reduce radiodermatitis occurring with adjuvant RT for localised breast cancer after lumpectomy. Participants will be randomised in a 1:1:1 ratio into each of the three randomisation arms: vehicle, 4.7% GR1014-CG, and 2.4% GR1014CG. A total of 273 participants will be needed for accrual. Participants will be screened in each centre from the pool of patients with primary, localised breast cancer without metastases pT1--3, pN0-N1mi, M0, who have undergone breast-conserving surgical excision and require adjuvant RT (ultra hypofractionated regimen; 26 Gy in 5 fractions over 1 week). They will be approached for willingness to participate in the study when seen in routine outpatient visits (initial consultation visit of RT). The participants will be followed for up to 8 weeks after the last RT session. The study will progress in two steps: • Safety run-in (in 3 designated investigational centres): ~30 participants to allow for ~8-10 evaluable participants per arm, i.e., all 30 participants will have completed the study with up to 8 weeks follow-up after the last RT treatment. The recruitment will be paused until Data Safety Monitoring Board (DSMB) decision. Pre-specified early stopping rules will be defined in a DSMB charter to allow for a possible halt of the trial or one of the active arms if the product or dosage investigated is deemed unsafe. The PK assessments and serum calcium level measurement will be collected in this group of participants. • After DSMB review and agreement, the recruitment will resume (all investigational centres) until the total of planned study accrual is reached. Study procedures will be as follows: **Visit 1** (V1) – Screening visit: up to 2 weeks before Visit 2.

	• Assessments: informed consent, collection of patient information, medical history, concomitant treatments, and planned RT protocol • Targeted physical examination and pregnancy test (if applicable) • Patient baseline characteristics (verification of dermatitis Grade 0 (CTCAE LV) and absence of other breast skin-relevant symptoms) before treatment initiation. • Verification that the subject meets inclusion/exclusion criteria • Skin colour a* and TEWL measurements, clinical photographs of relevant breast area (for subjects who consent) at one designated site only • DLQI questionnaire (ePRO) completed by participants • Blood sample (on safety run-in participants) **Visit 2** (V2) – Day 1, first session of RT: • Assessments: concomitant medications, targeted physical examination, vital signs (blood pressure [BP] and heart rate [HR]) • Pre-treatment verification of Grade 0 dermatitis (CTCAE LV) in the area to be irradiated • First treatment or placebo (vehicle gel) application, initial RT session • Pregnancy test should be repeated if the screening visit is performed >10 days from first dose administration • Blood sample (on safety run-in participants) if not done at Visit 1. **Visits 3, 4 and 5** (V3, V4 and V5) – Days 2 to 4 of RT: • Participants will receive study treatment or a placebo (vehicle gel) under supervision • Vital signs (HR and BP) will be measured before and after treatment application **Visit 6** (V6) – Day 5, final RT session:

	• Participants will receive study treatment or a placebo (vehicle gel) under supervision • Vital signs (HR and BP) will be measured before and after treatment application • Assessments: Evaluation of AEs, radiodermatitis, medications, and overall health • Blood samples (on safety run-in participants) • Skin colour a* and TEWL measurements, clinical photograph (at one designated site only) • DLQI questionnaire (ePRO) completed by participants • Targeted physical examination **Visits 7 up to 12** (V7 up to V12) – Follow-up visits for up to 8 weeks after the last RT session: • Assessments: Evaluation of AEs, radiodermatitis, medications, and overall health • Skin colour a* and TEWL measurement, Clinical photographs (at one designated site only) • DLQI questionnaire (ePRO) completed by participants • Targeted physical examination • For Visit 10 only, pregnancy test for participants of childbearing potential **End-of-Study (EOS) visit:** Visit 10 (4 weeks after the last RT session) will be the end of study visit for participants with radiodermatitis Grade 0 (CTCAE LV). If participants have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 10, they will perform an additional visit 6 weeks after the last RT session (Visit 11). The EOS visit will be Visit 11 for participants with radiodermatitis Grade 0 (CTCAE LV). If participants have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 11, they will perform an additional visit 8 weeks after the last RT session (Visit 12). **Early termination visit:**

	The same assessments and procedures as the EOS visit will be performed. During the study, from first to last visit, participants will assess pain and pruritus using 0-10 NRS (ePRO) every day. DLQI questionnaire (ePRO) will be completed by the participants at the time of Visit 1, or Visit 2 if not done at Visit 1 (baseline), and Visit 6 up to Visit 12. <u>Committees</u> • Data and Safety Monitoring Board (DSMB): Responsible for monitoring participant safety throughout the study. • Steering Executive Committee: Ensures adherence to the protocol, ethical considerations, and safety of participants. • Quality Assurance Committee for RT (RT-QA Committee): Ensures consistency in RT treatment procedures, reviews deviations, and offers support to investigators.
Number of participants planned	It is estimated that 273 participants will be enrolled. Assuming 10% of non-evaluable participants, 246 participants in total will be fully evaluable (82 participants per arm).
Statistical considerations	**Primary Efficacy Endpoint Analysis** Analysis will be performed on modified intent to treat (mITT) population and on a defined per protocol (PP) population as supportive analyses. The rate of participants with no radiation dermatitis (grade = 0, CTCAE LV) in the higher dosage active treatment group will be compared with the rate in the vehicle group using a Mantel-Haenszel chi-square test (considering the stratification factors bra cup size and previous chemotherapy), and a two-sided 95% confidence interval for the difference in these rates will be calculated. Upon rejection of the tested null hypothesis, the rate of participants with no radiation dermatitis in the lower dosage active treatment group will be compared with the rate in the vehicle group with the same methodology, providing a two-sided 95% confidence interval for the difference in these rates.

	Secondary and Exploratory Endpoint Analyses Efficacy Analyses will be performed on mITT population and on PP population as supportive analyses. Any discrepancies between the analyses will be carefully explored. The same methodology (including the hierarchical structure) of a Mantel-Haenszel chi-square test and two-sided 95% confidence intervals for the difference of rates will be used to analyse the following secondary endpoints: percentage of participants with grade ≥2 radiation dermatitis (CTCAE LV), percentage of participants with a severe radiodermatitis (CTCAE LV grade ≥ 3), and percentage of participants with no radiation dermatitis (CTCAE LV, grade of 0), 4 weeks after the last RT session (at Visit 10). Repeated measures analyses will be conducted on the assessed secondary endpoints of radiation dermatitis grades, skin colour values, pain score, TEWL and DLQI response variables, modelled using treatment arm and time point of assessment as classification variables with interaction effects. The endpoints duration of radiodermatitis grade ≥ 2 (CTCAE LV) and the time to onset of such will be analysed using survival analysis methodology. All safety and tolerability endpoints, as well as the maximum grade of radiodermatitis reached in each participant, will be analysed descriptively only. Analyses will include tabulation of the type (using the MedDRA classification) and frequency of all adverse events, as well as events considered by the investigator or by the sponsor to be at least possibly product-related. If possible (i.e., if methodology and/or sample size allows), all analyses will also be executed considering the following additional factors as covariates: • BMI < 25.1 vs. ≥ 25.1 (expressed in kg/m^2) • Postmenopausal vs premenopausal • Current tobacco use (chewing, smoking) (yes vs no) • Diabetes (yes vs no).

	Analysis Populations The modified Intent-To-Treat (mITT) population is composed of all randomised participants having received at least one application of the study treatment. The Per Protocol (PP) population is composed of all randomised participants having received at least one application of the study treatment, the full radiotherapy course and without any major protocol deviations. The safety population is composed of all participants having received at least one application of the study treatment. The PK population is composed of all randomised participants having had blood drawn at the defined time points prior to and post receiving the study treatment. **Sample Size** The initial hypothesis for sample size determination is the following: - For an estimated difference of at least 20% between 2 groups (30% in the GR1014-CG group and 10% in the vehicle group) in the primary endpoint, a minimum of 164 evaluable participants are required (82 participants per treatment group) for a power of 90% (1-β) and 5% risk of type I error (α) in a two-tailed Pearson's Chi-square test. - Given the design of 3 parallel groups (2 dosages of GR1014-CG and vehicle gel), 246 evaluable participants are required - Assuming 10% of participants are non-evaluable (dropouts, major deviations from the protocol, etc.), ~ 273 participants will need to be included (91 per treatment group).

2 SCHEDULES OF ACTIVITIES

Table 1 - Schedule of Activities for all Centres

Tests and procedures	Screening visit	First session of RT	RT session 2	RT session 3	RT session 4	Last session of RT	Follow-up			Early termination
Visit	Visit 1 (V1)	Visit 2 (V2)	Visit 3 (V3)	Visit 4 (V4)	Visit 5 (V5)	Visit 6 (V6)	Visit 7 (V7) to Visit 10 (V10)	Visit 11 (V11)[2]	Visit 12 (V12)[2]	
timepoints	Up to 2 weeks before V2	Day 1	Day 2[1]	Day 3[1]	Day 4[1]	Day 5[1]	Weekly for 4 weeks (7 ± 2 days)	6 weeks ± 2 days after the last RT session	8 weeks ± 2 days after the last RT session	
Informed consent	X									
Demography and anthropometric characteristics, including skin phototype, bra cup size, weight/height[3], menopausal status, tobacco use	X									
Medical history and breast cancer status	X									
RT protocol to be followed by the participant (3D or IMRT/VMAT, laterality, total prescribed dose [Gy], number of planned fractions)	X									
Calculated volume of IMP to be applied at each RT session (mL)	X									
Assessment of absence of pre-existing dermatitis signs in treatment area (CTCAE LV grade 0)	X	X								
Pregnancy test for participants of childbearing potential	X	X[4]					X			X
Verification of inclusion and exclusion criteria	X									

Tests and procedures	**Screening visit**	**First session of RT**	**RT session 2**	**RT session 3**	**RT session 4**	**Last session of RT**	**Follow-up**			**Early termination**
Visit	**Visit 1 (V1)**	**Visit 2 (V2)**	**Visit 3 (V3)**	**Visit 4 (V4)**	**Visit 5 (V5)**	**Visit 6 (V6)**	**Visit 7 (V7) to Visit 10 (V10)**	**Visit 11 (V11)[2]**	**Visit 12 (V12)[2]**	
timepoints	**Up to 2 weeks before V2**	**Day 1**	**Day 2[1]**	**Day 3[1]**	**Day 4[1]**	**Day 5[1]**	**Weekly for 4 weeks (7 ± 2 days)**	**6 weeks ± 2 days after the last RT session**	**8 weeks ± 2 days after the last RT session**	
Randomisation and kit number allocation for eligible participants	X									
Investigational treatment										
Application of GR1014-CG or vehicle gel 15-30 min before irradiation		X	X	X	X	X				
Date, time of application, time of cleansing, kit number, and volume of gel applied		X	X	X	X	X				
Radiation therapy sessions										
Date and start time of the RT session and fraction dose delivered (Gy)		X	X	X	X	X				
Efficacy										
Assessment of dermatitis and other AEs[5] linked to RT (CTCAE LV)						X	X	X	X	X
Daily reported worst pain intensity (010 NRS)	X	X	X	X	X	X	X	X	X	X
Daily reported peak pruritus intensity (0-10 NRS)	X	X	X	X	X	X	X	X	X	X
DLQI (participant auto-evaluation)	X[6]	X[6]				X	X	X	X	X
Safety										
Targeted physical examination, performance status	X	X				X	X	X	X	X
Previous and concomitant treatments	X	X				X	X	X	X	X

Tests and procedures	Screening visit	First session of RT	RT session 2	RT session 3	RT session 4	Last session of RT	Follow-up			Early termination
Visit	Visit 1 (V1)	Visit 2 (V2)	Visit 3 (V3)	Visit 4 (V4)	Visit 5 (V5)	Visit 6 (V6)	Visit 7 (V7) to Visit 10 (V10)	Visit 11 (V11)[2]	Visit 12 (V12)[2]	
timepoints	Up to 2 weeks before V2	Day 1	Day 2[1]	Day 3[1]	Day 4[1]	Day 5[1]	Weekly for 4 weeks (7 ± 2 days)	6 weeks ± 2 days after the last RT session	8 weeks ± 2 days after the last RT session	
AEs, SAEs		X	X	X	X	X	X	X	X	X
Vital signs (heart rate, blood pressure)[7]		X	X	X	X	X				

Table 2 - Schedule of Additional Activities at one Designated Investigational Centre

Tests and procedures	Screening visit	First session of RT	RT session 2	RT session 3	RT session 4	Last session of RT	Follow-up			Early termination
Visit	Visit 1 (V1)	Visit 2 (V2)	Visit 3 (V3)	Visit 4 (V4)	Visit 5 (V5)	Visit 6 (V6)	Visit 7 (V7) to Visit 10 (V10)	Visit 11 (V11)[2]	Visit 12 (V12)[2]	
timepoints	Up to 2 weeks before V2	Day 1	Day 2[1]	Day 3[1]	Day 4[1]	Day 5[1]	Weekly for 4 weeks (7 ± 2 days)	6 weeks ± 2 days after the last RT session	8 weeks ± 2 days after the last RT session	
Efficacy										
Skin colour a* value	X[6]	X[6]				X	X	X	X	X
TEWL	X[6]	X[6]				X	X	X	X	X
Clinical photo of the treatment area[8]	X[6]	X[6]				X	X	X	X	X

Table 3 - Schedule of Additional Activities for Safety run-in Participants

Tests and procedures	Screening visit	First session of RT	RT session 2	RT session 3	RT session 4	Last session of RT	Follow-up	Early termination
Visit	Visit 1 (V1)	Visit 2 (V2)	Visit 3 (V3)	Visit 4 (V4)	Visit 5 (V5)	Visit 6 (V6)	Visit 7 (V7) up to visit 12 (V12)	
timepoints	Up to 2 weeks before V2	Day 1	Day 2[1]	Day 3[1]	Day 4[1]	Day 5[1]		
Blood sample for serum calcium	X[6]	X[6]				X[9]		
Blood sample for PK analysis						X[10]		

Footnote for Table 1, Table 2 and Table 3:

1. An overall delay of 3 days for temporary interruption(s) of the RT protocol will be tolerated
2. Visit 11 and Visit 12 are applicable for participants who have radiodermatitis higher than Grade 0 (CTCAE LV) at the previous visit, i.e., patients who have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 10 will perform an additional visit 6 weeks after the last RT session (Visit 11) and patients who have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 11 will perform an additional visit 8 weeks after the last RT session (Visit 12)
3. BMI and BSA will be automatically calculated using weight and height collected at screening
4. The pregnancy test should be repeated at V2 if the screening visit is performed > 10 days prior to the administration of the first dose of the study treatment.
5. Oedema; hyperpigmentation; skin infection; need for topical and systemic antibiotics; need for topical steroids; need for analgesics; need for silicone-based dressings
6. To be performed ≤2 weeks before treatment initiation, at V1 or V2 (baseline).
7. Vital signs will be measured from Day 1 to 5 before and after each RT session (V2 to V6)
8. Optional. Only for participants who provide a specific written consent
9. Collected pre-dose and post-dose at 2.5 h
10. Collected pre-dose and post-dose at 45 min, 1.5 h, 2.5 h

Abbreviations: AE=Adverse Event; CTCAE LV=Common Terminology Criteria for Adverse Events latest version; BMI=Body Mass Index; BSA=Body Surface Area; DLQI=Dermatology Life Quality Index; 3D=3 Dimensions; Gy=Gray; IMRT= Intensity-modulated radiotherapy; NRS: numerical rating scale; PK=Pharmacokinetics; RT=Radiation Therapy; SAE=Serious Adverse Event, TEWL=Transepidermal Water Loss; V1=Visit 1; V2=Visit 2 etc.; VMAT= Volumetric modulated arc therapy

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LIST OF ABBREVIATIONS

Term	Description
ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Event of Special Interest
BMI	Body Mass Index
BSA	Body Surface Area
CI	Confidence Interval
CIELAB	Colour space L*a*b* defined by the Commission Internationale de l'Eclairage in 1976
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CRA	Clinical Research Assistant
CTCAE LV	Common Terminology Criteria for Adverse Events latest version
CTVp breast	Breast Clinical Target Volume
DCIS	Ductal Carcinoma in Situ
DLQI	Dermatology Life Quality Index
DVH	Dose-Volume Histogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
ePRO	Electronically captured Patient Reported Outcome(s)
EQD2	Equivalent dose in 2Gy fractions
ER	Estrogen Receptor
FIH	First in Human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GR1014-CG	G1014 cutaneous gel
Gy	Gray
HER-2	Human epidermal growth factor receptor-2
IB	Investigator’s Brochure
ICH	International Conference on Harmonization

Term	Description
ICF	Inform Consent Form
IDSMC	Independent Data and Safety Monitoring Committee
IEC	Institutional Ethics Committee
IV	Intravenous
IRB	Institutional Review Board
IMP	Investigational Medical Product
IWRS	Interactive Web Response System
LC/MS	Liquid Chromatography / Mass Spectroscopy
MedDRA	Medical Dictionary for Regulatory Activities
NA	Not Applicable
NCI	National Cancer Institute
mITT	Modified Intent-To-Treat
NRS	Numerical Rating Scale
OAR	Organs At Risk
PK	Pharmacokinetic
PP	Per-protocol
PR	Progesterone Receptor
PTVp breast	Breast Planning Target Volumes
RT	Radiation Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SD	Standard Deviation
SoA	Schedule of Activities
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
TEWL	Transepidermal Water Loss
TLD	Thermo-Luminescent Dosimetry
UAE	Unexpected Adverse Event
UAR	Unexpected Adverse Reaction

3 INTRODUCTION

3.1 Background

Breast cancer, the most common form of cancer in women, is frequently managed through the surgical removal of the tumour followed by adjuvant radiation therapy (RT) to decrease the chances of local recurrence and improve survival rates. Several randomised trials have demonstrated that RT significantly reduces the rates of recurrence and cancer-related mortality in early breast cancer patients. However, despite its benefits, RT can result in both short-term and long-term side effects.

One significant side effect is radiation dermatitis, a distressing condition caused by inflammation, cell necrosis, and cell death, along with changes in endothelial cells due to tissue damage. Symptoms of radiation dermatitis include skin redness (erythema), swelling (oedema), dry or moist peeling of the skin (desquamation), and varying degrees of pain, ranging from mild to severe or incapacitating. These symptoms usually appear within 1 to 4 weeks of starting radiation and may persist or worsen during or after treatment but typically improve and heal about 2 to 4 weeks after completing RT.

Radiation dermatitis can have a significant impact on a patient's quality of life, and in severe cases, it may even lead to interruptions or discontinuation of treatment. Certain patient factors, such as smoking status and larger breast size, as well as treatment-related factors, like the use of bolus for RT and concurrent chemotherapy, can increase the risk of developing radiation dermatitis.

While patients have access to comfort measures like pain relievers and topical creams, evidence suggests that the severity of radiation dermatitis peaks about 1 to 2 weeks after the last RT session. The study conducted by L. Drost et al. provides additional evidence that radiation dermatitis should continue to be closely followed, especially in the 2 weeks following RT.[1]

Amifostine is a prodrug that exerts its effects through its active metabolite, amifostine thiol (GR1014). The conversion happens through dephosphorylation mediated by alkaline phosphatase. The cytoprotective properties of amifostine thiol are potentially beneficial in preventing radiodermatitis during RT, particularly in breast cancer patients.

3.2 Study rationale

The involvement of reactive oxygen species in the development of radiodermatitis suggests that reducing their production or scavenging them from tissue could be a potential strategy for prevention. Aminothiols combine the radioprotective properties

of thiols with the ability of amines and polyamines to protect DNA from radiation damage.[2]

Amifostine belongs to this group of compounds and was originally developed for its potential to protect normal tissues against damage caused by ionizing radiation.[3] Amifostine is a prodrug converted to the active metabolite, amifostine thiol (GR1014, WR1065), which acts as a nonspecific cytoprotectant, reducing the toxicity associated with alkylating agents and RT by scavenging free radicals.

Amifostine (Ethyol®, a sterile lyophilised powder for IV infusion) received approval in Australia, the European Union (EU), and the United States (US) for use i) in chemotherapy to reduce renal toxicity associated with repeated cisplatin administration and the neutropenia-related risk of infection induced by cyclophosphamide and/or cisplatin in advanced ovarian cancer and ii) in RT to reduce the incidence of xerostomia in patients undergoing post-operative radiation treatment for head and neck cancer.

Currently, Ethyol® is marketed in Australia and the US for the following specific indications:

Australia label

- To decrease the incidence of neutropenia-related fever and infection induced by DNA-binding chemotherapeutic agents (classical alkylating agents such as cyclophosphamide and non-classical alkylating agents such as mitomycin-C and platinum-containing drugs); decrease the incidence of acute and cumulative nephrotoxicity associated with platinum-based therapy; and to provide better adherence to these types of chemotherapy regimens.
- To protect against acute and late xerostomia associated with standard fractionated RT in patients with head and neck cancer.

US label

- To reduce cumulative renal toxicity associated with repeated administration of cisplatin in patients with advanced ovarian cancer.
- To reduce the incidence of moderate to severe xerostomia in patients undergoing post-operative radiation treatment for head and neck cancer, where the radiation port includes a substantial portion of the parotid glands.

However, there are significant barriers to using amifostine as a radioprotector to prevent or reduce radiodermatitis in breast cancer patients. The logistics of IV administration are impractical for outpatients undergoing RT, making it a less desirable option for patient acceptance and compliance. Moreover, it causes high rates of severe orthostatic hypotension, rash, nausea, and vomiting.

To address these challenges, GR1014 cutaneous gel (GR1014-CG) is designed to be topically applied to the skin before each RT session, delivering a sufficient concentration of the active metabolite amifostine thiol in the epidermis to achieve its radioprotective effect while limiting systemic exposure, thereby reducing the risk and seriousness of systemic adverse events (AEs).

This is a dose-finding, phase-2 trial to investigate the safety, tolerability, and efficacy of GR1014-CG as a topical radioprotector in the reduction of radiodermatitis occurring in patients with adjuvant ultra-hypofractionated RT (26 Gy in 5 fractions over 1 week)[4,5] for localised, non-metastatic breast cancer after lumpectomy versus vehicle gel.

The 26-Gy/5-fraction regimen has been chosen in this study as a prototypical RT example for the demonstration of the preventive effect of GR1014-GC on acute radiation dermatitis (proof-of-concept). Assessment of the benefits of the product in other RT regimens, hypofractionated and conventional, will be performed in following studies.

As this is the first-in-human (FIH) use of GR1014-CG, two dosages will be investigated, to also allow more insight into the peak plasma exposure. The efficacy objectives will focus on the higher dosage, while the lower dosage will also help to understand the safety profile.

Up to 8 weeks follow-up of the participants is planned. The signs and symptoms of radiodermatitis typically appear within 1 to 4 weeks of starting radiation and may persist or worsen during the first week after treatment. They usually improve and heal about 2 to 4 weeks after completing RT. Eight (8) weeks follow-up in total after completing RT should be long enough to capture the peak of acute toxicity and toxicity healing.

4 STUDY OBJECTIVES, ENDPOINTS

4.1 Objectives

4.1.1 Primary Objectives

To investigate the safety, tolerability, and efficacy of GR1014-CG (4.7%; 2.4%) as a treatment to prevent radiodermatitis occurring with adjuvant ultra hypofractionated RT for localised, non-metastatic breast cancer after lumpectomy versus vehicle gel.

4.1.2 Secondary Objectives

1. To assess the efficacy of GR1014-CG (4.7%; 2.4%)
   - in reducing radiodermatitis severity versus vehicle gel.
   - in reducing peak radiodermatitis severity versus vehicle gel.
   - in reducing radiodermatitis emergence versus vehicle gel.

- in delaying the onset of radiodermatitis versus vehicle gel.
- in reducing the duration of radiodermatitis versus vehicle gel.
- in reducing severe radiodermatitis versus vehicle gel.
- in reducing the side effects of RT other than dermatitis versus vehicle gel.
- in reducing patient-reported pain intensity in the irradiated skin region induced by RT versus vehicle gel.
- in reducing patient-reported pruritus intensity in the irradiated skin region induced by RT versus vehicle gel.
- on participants' assessment of their quality of life during and after RT versus vehicle gel.

2. To characterise the plasma C_{max} of amifostine thiol after application of GR1014-CG.

4.1.3 Exploratory Objectives

1. To assess the efficacy of GR1014-CG in reducing skin hyperaemia during and after RT versus vehicle gel.
2. To assess the ability of GR1014-CG in preventing the loss of integrity of the skin barrier function during and after RT versus vehicle gel.

4.2 Study Endpoints

4.2.1 Primary efficacy endpoint

The primary efficacy endpoint is the percentage of participants with no radiation dermatitis (Common Terminology Criteria for Adverse Events latest version [CTCAE LV] grade of 0) between the first RT session and 4 weeks after the last one.

Justification: When compared to the vehicle group, the population group treated by GR1014 CG is expected to present a higher percentage of participants without radiation dermatitis.

4.2.2 Secondary efficacy endpoints

- Percentage of participants with a radiodermatitis grade ≥2 (CTCAE LV) occurring at any time between the first RT session and 4 weeks after the last one – key secondary.
- Percentage of participants with no radiation dermatitis (CTCAE LV, grade of 0), 4 weeks after the last RT session (at Visit 10)
- Maximum grade of radiodermatitis (CTCAE LV) reached in each participant between the first RT session and 4 weeks after the last one.
- Assessments of radiodermatitis grades (CTCAE LV) between the first RT session and the last visit.
- Time to onset of radiodermatitis grade ≥2 (CTCAE LV).
- Duration of radiodermatitis grade ≥2 (CTCAE LV).

- Percentage of participants with severe radiodermatitis (grade ≥3; CTCAE LV) occurring at any time between the first RT session and 4 weeks after the last one.
- Number of cases of side effects of RT other than radiodermatitis occurring at any time between the first RT session and the last visit (oedema; hyperpigmentation; skin infection; need for topical and systemic antibiotics; need for topical steroids; need for analgesics; need for silicone-based dressings)

Patient Reported Outcome assessments

- Absolute change from baseline in weekly averaged worst skin pain score in the irradiated area. Worst pain intensity (during the last 24 hours) scored daily on a 0--10 NRS, from first to last visit.
- Absolute change from baseline in weekly averaged peak pruritus score in the irradiated area. Peak pruritus intensity (during the last 24 hours) scored daily on a 0-10 NRS, from first to last visit.
- Total score and sub-scores of DLQI addressed, from the first RT session and to the last visit.

Justification: The DLQI is a simple, self-administered and user-friendly validated questionnaire designed to measure the health-related quality of life of adult participants suffering from a skin disease. DLQI has been used in many different skin conditions in over 80 countries and is available in over 110 translations. It is the most frequently used patient-reported outcome measure in randomised controlled trials in dermatology.

4.2.3 Exploratory endpoints (at a designated investigational site)

- Breast skin surface inflammation evolution will be assessed through a repeated measures analysis of the skin colour a* values calculated using a tristimulus colorimetric instrument in accordance with the CIE L*a*b* colour space (CIELAB) colour systems from the first RT session to the last visit, in the same 4 test areas on the breast as for the TEWL.

Justification: Within CIELAB, an increased a value means that redness increases and is then a good marker of skin erythema.*

- TEWL measured from the first RT session to the last visit for 4 test areas within the radiation field of the breast (1 site per quadrant, including the inframammary fold for the internal and external inferior quadrants) and on a control area of the volar aspect of the forearm on the non-irradiated side.

Justification: TEWL measurement is a good indicator of the integrity of the skin barrier function, which inherently refers to the skin's ability to retain moisture. An increase in the TEWL indicates an impaired barrier function. The inframammary fold is the most frequent site of radiodermatitis when delivering breast radiation therapy because of its concavity, possible dose heterogeneity and possible rubbing when pendular breasts.

The last visit may be at 4 weeks, 6 weeks or 8 weeks after the last RT session, depending on subject's attainment of radiodermatitis Grade 0 (CTCAE LV).

4.2.4 Safety endpoints

- Serious Adverse Reactions (SARs)
- Adverse Events of special interest (AESIs†)
- Percentage of study withdrawal
- Stop/delay in RT sessions due to safety events.

†The side effects of the RT (radiodermatitis, oedema, hyperpigmentation, skin infection, need for topical and systemic antibiotics, need for topical steroids, need for analgesics, need for silicone-based dressings) will be excluded from the safety assessments since they are assessed as efficacy endpoints.

The AESIs for this protocol have been pre-defined as follows:

- Nausea and vomiting are common reactions associated with amifostine (ETHYOL®) IV administration. Although the amifostine thiol plasma levels at which these adverse events occur are unlikely to be reached after topical cutaneous application of GR1014-CG, in case of nausea or vomiting, treatment will be left at the investigator's choice according to symptoms and severity.
- Hypotension is a common reaction in patients treated with amifostine IV (ETHYOL®) linked to amifostine thiol plasma levels. Although the amifostine thiol plasma levels associated with hypotension are unlikely to be reached after topical cutaneous application of GR1014-CG, in case of hypotension, treatment will be left at the investigator's choice according to symptoms and severity.
- Hypocalcaemia has been associated with ETHYOL® IV administration. Although the expected circulating levels of amifostine thiol after topical cutaneous application of GR1014-CG are predicted to be far lower than after IV administration of amifostine, in case of hypocalcaemia treatment will be left at the investigator's choice according to symptoms and severity.
- Cutaneous reactions. Rare but severe cutaneous reactions have been associated with repeated ETHYOL® (amifostine) IV administration. Cutaneous reactions must be clearly differentiated from radiation-induced dermatitis and cutaneous reactions related to an alternate aetiology. For cutaneous reactions that appear outside the application site or outside the radiation port without a known aetiology, GR1014-CG should be withheld, and dermatologic consultation and biopsy should be considered to classify the reaction. The cutaneous reaction treatment will be left at the investigator's choice according to symptoms and severity. Reinitiation of IMP administration should be at the investigator's discretion based on medical evaluation and appropriate dermatologic consultation.

- Hypersensitivity reactions. In case of severe acute allergic reactions, GR1014-CG should be immediately and permanently discontinued.

4.2.5 Pharmacokinetics assessments

- Observed maximum of plasma concentration (C_{max})
- Time to C_{max} (T_{max})
- Area under the plasma concentration time curve from time 0 to the last measurable time point t_{last} (AUC_{last})

5 STUDY PLAN

5.1 Overall Study Design

This is an interventional, vehicle-controlled, multi-centre, double-blinded, parallel-group trial in participants to assess the ability of GR1014-CG to prevent or reduce radiodermatitis occurring with adjuvant radiotherapy for localised breast cancer after lumpectomy.

Participants will be randomised in a 1:1:1 ratio into each of the three randomisation arms: vehicle, 4.7% GR1014CG, and 2.4% GR1014CG. A total of 246 participants, 82 participants in each of the 3 randomisation arms, will be needed for the analysis. Assuming a drop-out rate of 10%, 273 participants will be included over approximately 10 months in 10 centres and randomised in 3 parallel groups (two GR1014 CG doses, vehicle gel).

Participants will be screened in each centre from the pool of patients with primary, localised breast cancer without metastases pT1--3, pN0-N1mi, M0, who have undergone breast-conserving surgical excision and require adjuvant RT (ultra hypofractionated regimen; 26 Gy in 5 fractions over 1 week). They will be approached for willingness to participate in the study when seen in routine outpatient visits (initial consultation visit of RT).

5.2 Study Periods and Visits

5.2.1 Study periods

The study will progress in two steps (Figure 1):

Safety run-in (in 3 designated investigational centres): ~30 participants to allow for ~8-10 evaluable participants per arm, i.e., all 30 participants will have completed the study with up to 8 weeks follow-up after the last RT treatment. The recruitment will be paused until Data Safety Monitoring Board (DSMB) decision. Pre-specified early stopping rules defined in a separate DSMB charter to allow for a possible halt of the

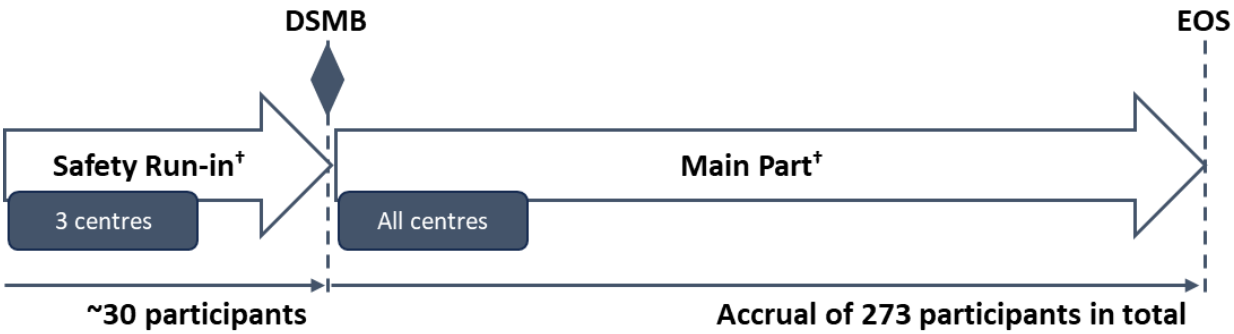
complete trial or one of the active arms if the product or dosage investigated is deemed unsafe.
The PK assessments and serum calcium level measurement will also be done in this group of participants.

Main part (all investigational centres): after DSMB review and agreement, the recruitment will resume until the total of planned study accrual is reached.

A participant is considered to have completed the study if he/she has completed all phases of the study including follow-up.

The end of the study is defined as the date of the last visit of the last participant in the study.

Figure 1 - Study periods

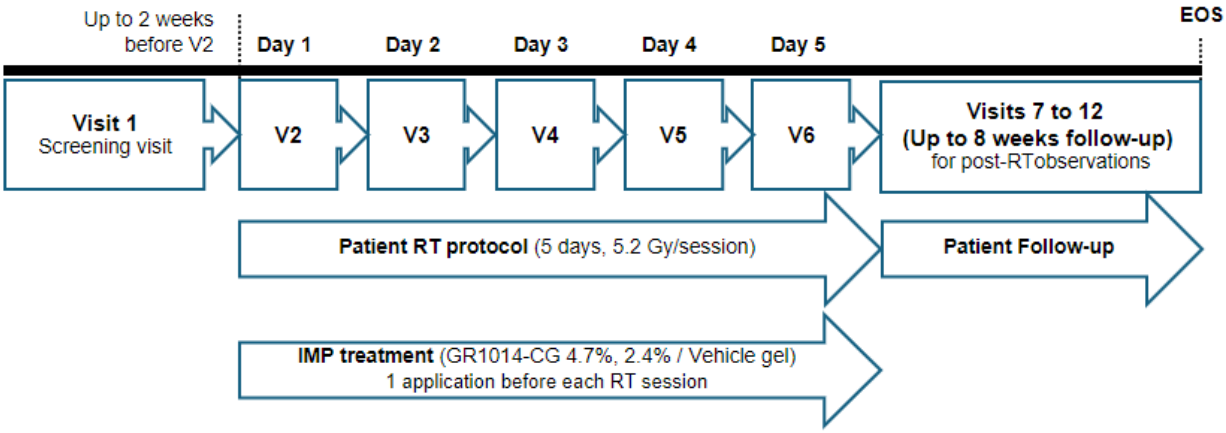


†Exploratory Assessments (colour a*, TEWL and photos) to be performed at a single designated centre

5.2.2 Study visits

The study duration and study visits are described in Figure 2.

Figure 2 - Study visits



Study procedures will be as follows:

- **<u>Visit 1 (V1) – Screening visit</u>**, to be done up to 2 weeks before V2

  - Informed consent
  - Demography and anthropometric characteristics, including skin phototype, bra cup size, weight, height, menopausal status, and tobacco use
  - Cancer stage (IA-IIIB), histological type, and grade (1-3), ER and PR status, HER2/neu status
  - Chemotherapy, endocrine therapy adjuvant/neoadjuvant (Yes/No),
  - Previous oncologic treatments: type of surgery, chemotherapy adjuvant/neoadjuvant (Yes/No), immunotherapy (Yes/No)
  - Medical history
  - Concomitant treatments (including oncologic and non-oncologic treatments, pain killers, homoeopathic treatment and possible non-medical treatments/devices)
  - Radiotherapy protocol to be followed by the participant
  - Targeted physical examination and performance status
  - Assessment of potential pre-existing signs of dermatitis in the breast area to be irradiated. Verification of Grade 0 as per CTCAE LV radiation dermatitis grading and absence of other breast skin relevant symptoms) before treatment initiation.
  - Urine pregnancy test for participants of childbearing potential
  - Verification that the subject meets inclusion/exclusion criteria
  - Subject identifier assignment

Participants will be equipped with ePROs for the daily assessment of pain and pruritus, using a 0-10 NRS. They will complete the first evaluation at the centre during the screening visit and will complete the following daily evaluations, until the End-of-Study visit (Visit 10, Visit 11 or Visit 12 depending on the grade of radiodermatitis).

<u>Randomisation</u>

- Verification of inclusion and non-inclusion criteria

- Randomisation and kit number allocation
- Baseline determination, ≤2 weeks before treatment initiation (to be done at V1 or V2):
  - DLQI (ePRO)
  - Skin colour a* value (at one designated investigational centre)
  - TEWL (at one designated investigational centre)
  - Clinical photographs of the to-be-treated area (at one designated investigational centre)
  - Blood sampling according to procedure (on safety run-in participants only)

- **Visit 2 (V2) – Day 1, first session of radiotherapy**

  The participant arrives at the centre at least 1 hour before the RT session and is evaluated as follows:

  - Concomitant medications taken since the last visit
  - Targeted physical examination and performance status
  - Pre-treatment verification of absence of dermatitis signs in the area to be irradiated (CTCAE LV Grade 0)
  - Urine pregnancy test should be repeated if the screening visit is performed >10 days from first dose administration.
  - Blood sampling according to procedure (on safety run-in participants only) if not done at Visit 1
  - First study treatment application by the participant under the supervision of the investigational centre health care personnel 15-30 min before RT
  - Vital signs (heart rate and blood pressure [HR and BP]) will be measured before treatment application and after the RT session.

- **Visits 3, 4, and 5 (V3, V4 and V5) – Day 2 to Day 4 of Radiotherapy sessions 2 to 4:**

  - Study treatment application by the participant under the supervision of the investigational centre health care personnel 15-30 min before RT
  - Vital signs (HR and BP will be measured before treatment application and after the RT session.

- **Visit 6 (V6) – Day 5‡, final RT session**

  - Vital signs (HR and BP) will be measured before treatment application and after the RT session
  - CTCAE and other side-effects of RT†
  - DLQI (ePRO)
  - Concomitant medications taken since the last visit
  - Targeted physical examination, and performance status
  - Safety (AEs, SAEs)

- Skin colour a* (at one designated investigational centre)
- TEWL (at one designated investigational centre)
- Clinical photographs of the treated area (at one designated investigational centre)
- Blood sampling according to procedure (on safety run-in participants only) will be collected at the following time points (pre-dose; post-irradiation, i.e., 45 min post-dose; 1.5 h post-dose; 2.5 h post-dose)

- **<u>Visits 7 up to to 12 (V7 to V12) – Follow-up visits for up to eight (8) weeks after the last RT session</u>**

  - CTCAE and other side-effects of RT†
  - DLQI (ePRO)
  - Concomitant medications taken since the last visit
  - Targeted physical examination and performance status
  - Safety (AEs, SAEs)
  - For V10 only, pregnancy test for participants of childbearing potential
  - Skin colour a* (at one designated investigational centre)
  - TEWL (at one designated investigational centre)
  - Clinical photographs of the treated area (at one designated investigational centre)

†*Evaluation of radiation dermatitis according to CTCAE LV, and other side-effects of RT (oedema, hyperpigmentation, skin infection, need for topical and systemic antibiotics, need for topical steroids, need for analgesics, need for silicone-based dressings)*

‡*May be as late as Day 8, an overall delay of 3 days for temporary interruption(s) of the RT protocol will be tolerated*

Clinically significant changes from baseline in relation to serum calcium values, vital signs and other clinically significant abnormalities should be recorded as AEs.

Adverse events (AEs) will be recorded beginning with the signature of the ICF and will continue through the last study visit. Concomitant medications will be recorded throughout the study.

The participant will be treated for 5 days (before each RT session) and will be followed for up to 8 weeks after termination of radiotherapy, leading to a total study duration per participant of approximately 10 weeks.

<u>End-of-Study (EOS) visit:</u>

Visit 10 (4 weeks after the last RT session) will be the end of study visit for the participants with radiodermatitis Grade 0 (CTCAE LV).

If participants have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 10, they will perform an additional visit 6 weeks after the last RT session (Visit 11). The EOS visit will be Visit 11 for participants with radiodermatitis Grade 0 (CTCAE LV).

If participants have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 11, they will perform an additional visit 8 weeks after the last RT session (Visit 12).

Early termination visit: the same assessments and procedures as the one of the EOS visit will be performed.

6 STUDY POPULATION

6.1 Inclusion Criteria

A potential study participant must meet all of the following inclusion criteria to be eligible to be included in the study:

1. Dated, signed informed consent obtained from patients who agree to participate in the study.
2. Female patients with age ≥18 years.
   Those of childbearing potential must be using highly effective contraception methods during the study and for 30 days after the last administration of the study treatment and have a negative pregnancy test at screening and no more than 10 days prior to the administration of the first dose of study treatment.
   Note: A woman is considered of childbearing potential (WOCBP), i.e., fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
3. Patients with primary, localised breast cancer without metastases pT1-3, pN0-N1mi, M0, who have undergone breast-conserving surgical excision and require adjuvant RT. If adjuvant chemotherapy is delivered, the patient must be randomised within 4 weeks after the last series of adjuvant chemotherapy. If no adjuvant chemotherapy is delivered, the patient must be randomised within 8 weeks from the last surgery. The patient can be included no matter the status of oestrogen and progesterone receptors, malignancy grade, or HER2 status.
4. Eastern Cooperative Oncology Group (ECOG) performance status 0-2
5. Patients to be treated with ultra hypofractionated RT, 26 Gy in 5 fractions (5.2 Gy) on the whole breast (EQD_2 > 42.6 Gy for α/β of 3).[5]
6. Patients with no signs of dermatitis in the breast area to be irradiated, i.e., assessed Grade 0 as per CTCAE LV radiation dermatitis grading.
7. Patients whom the investigator has deemed able to comply with the RT and investigational treatment done under the supervision of the medical personnel throughout the study period.

8. Patients affiliated with the Social Security System (France).
9. Patients who have completed the appropriate washout period for any prior interventions or treatments.

Contraception
Female subjects must agree to use one or more methods of highly-effective contraception during the course of the trial and for 30 days after the last administration of the trial drug.

A highly effective birth control method is defined as one which can achieve a failure rate of less than 1% per year when used consistently and correctly. Such highly effective birth control methods include:

- Hormonal methods of contraception, including combined (estrogen and progestogen containing) hormonal contraception; progestogen-only hormonal contraception associated with inhibition of ovulation; intrauterine device (IUD); intrauterine hormone-releasing system (IUS);
- Non-hormonal methods of contraception, such as bilateral tubal occlusion and a vasectomized partner (on the understanding that this is the only one partner during the whole trial duration and that the surgical success of the vasectomy has been medically assessed).

Recommendations regarding methods of contraception may differ depending on cancer type (e.g., status of oestrogen and progesterone receptors).

6.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Pregnant and breastfeeding women.
2. Patients under any treatment concomitant to RT tested in another clinical study.
3. Allergies to any of the ingredients in GR1014-CG.
4. Patients protected by law (legal guardianship or protection).
5. Patients unable to adhere to the requirements of the study.
6. History of thoracic RT.
7. Participants with the presence of skin rash, ulceration, unhealed surgical wounds, biopsy sites, or open wounds in the breast or chest area at visit 2.
8. Patients suffering from scleroderma, auto-immune disease, micro-vascular diseases, collagen tissue diseases, lupus, pre-existing loss of skin integrity, active eczema in the region to be treated, or with a history of any of the following: drug-induced severe cutaneous adverse reaction (SCAR; including, but not limited to Stevens-Johnson syndrome/toxic epidermal necrolysis [SJS/TEN], or drug reaction with eosinophilia and systemic symptoms.

6.3 Treatment Considerations

Recommendations to participants during treatment

- At home, washing/skin cleansing with mildly warm water and neutral, gentle fragrance-free soap (preferably, a surgras soap) to avoid any interference with the study substances is asked during the entire study duration.
- Protect irradiated skin area from ultraviolet exposure (e.g., sun exposure, tanning beds).
- Minimise skin trauma from excessive movements (especially friction within the irradiated area), exposure to extreme temperatures, or adhesives.
- Avoid shaving or hair removal of the axilla on the treated side.
- Consider protective dressings for areas of moist desquamation.

Forbidden concomitant treatments

During the study period, certain treatments or interventions are strictly prohibited to ensure the accuracy and validity of the results. Participants must refrain from using the following concomitant treatments:

- Topical steroids (e.g., mometasone, betamethasone) in the treatment area.
- Nonsteroidal agents (e.g., silver sulfadiazine, calendula ointment, barrier films) in the treatment area.
- Radio-sensitizing drugs are not authorised during RT (importantly, their respective washout period must be considered).
- If, in case of any toxicity of grade ≥2, in the judgment of a participant's clinician, radiation dermatitis necessitated initiation of an agent other than the study medication, the participant will discontinue the study medication and continue with evaluations in accordance with the study protocol.
- Supporting skin cosmetics care: Any application of either cream, body lotion or other cosmetic, including deodorant, is forbidden on the treated zone/side during the entire study duration.
- For participants in the designated centre on whom TEWL will be measured, no topical treatment is allowed on the volar aspect of the forearm for 8 hours before TEWL measurement.

6.4 Screening Failures

A screening failure occurs when a participant who has consented to participate in the clinical trial is not subsequently assigned to trial intervention. A minimal set of screening failure information is required to ensure transparent reporting of screening failure participants to meet the CONSORT publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screening failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this trial (screening failure) may be rescreened if the reason for the screening failure is considered reversible (e.g., laboratory value). Rescreened participants should be assigned a new participant number for every screening/rescreening event.

6.5 Participant Withdrawal/Discontinuation Criteria

Participants are to be permanently withdrawn from the trial or trial-specified treatments for any of the following reasons:

- Withdrawal of informed consent
- Unacceptable toxicity or safety concerns
- Changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the investigator
- Participant non-compliance as assessed by the investigator and/or Sponsor
- Lost to follow-up
- Request from the participant to not receive further treatment
- Any medical event requiring the administration of prohibited concomitant treatment(s)
- Early trial termination by Sponsor for any reason

The reason for the participant’s permanent discontinuation of trial treatment prior to planned or withdrawal from the trial should be indicated in the electronic Case Report Form (eCRF), and supporting data should be included. The investigator will make every effort so that these participants perform an early discontinuation visit, according to the SoA.

Participants can voluntarily withdraw consent from participation in the trial for any reason at any time. In this case, they should notify the investigator of the decision to withdraw consent from future follow-up in writing, whenever possible.

If the participant withdraws consent for disclosure of future information, no further trial-specific evaluations should be performed, and no additional data will be collected. The Sponsor may retain and continue to use any data collected before such a withdrawal of consent, except in the case of participant opposition. Any opposition should be transmitted by the investigator to the Sponsor without undue delay, and the investigator must document this in the site trial records.

6.6 Lost To Follow-Up

If a participant does not return for a scheduled visit, every reasonable effort should be made to contact him/her.

The following actions must be taken if a participant fails to return to the site for a scheduled visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the trial.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, telephone calls, and if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). This includes follow-up with people authorised by the participant, if applicable. These contact attempts should be documented in the participant's medical record. In any circumstance, every effort should be made to document the participant outcome, and all attempts should be documented in the corresponding medical file.
- A participant will be considered lost to follow-up if it is not possible to reach the enrolled participant after a minimum of three documented phone calls, faxes, or emails as well as a lack of response by enrolled participants to one registered mail letter.

Site personnel, or an independent third party, will attempt to collect information on participant outcomes within legal and ethical boundaries. Public sources may be searched for vital status information. If it is determined that the participant has died, this will be documented, and the participant will not be considered lost to follow-up. Sponsor personnel will not be involved in any attempts to collect vital status information.

6.7 Stopping rules

6.7.1 Early Stopping Rules

The termination of the study or one of the arms with the investigational product during the run-in phase will be considered by the DSMB if 3 or more participants in either arm with GR1014-CG present with adverse events of CTCAE grade 4 that are assessed by the Investigator as possibly related to the investigational product.

6.7.2 Advancement to the Main Phase

At the end of the safety-run-in, DSMB can propose that one or both active arm(s) will proceed to the main phase if less than 30% of subjects experience dermatitis Grade ≥3.

7 STUDY ASSESSMENTS AND PROCEDURES

Detailed information on study assessments is provided in the below subsections. Study procedures and their timing are summarised in the SoA. Adherence to the

study design requirements, including those specified in the SoA, is essential and required for study conduct.

Safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue the study intervention.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management and obtained before signing the ICF may be utilised for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

7.1 CLINICAL AND PARACLINICAL ASSESSMENTS

7.1.1 Demography and anthropometric characteristics

Demography (age) and anthropometric characteristics (skin phototype, bra cup size, weight, height, menopausal status, and tobacco use) will be collected in the eCRF at the screening visit. Body Mass Index (BMI, kg/m^2) and Total Body Surface Area (TBSA, m^2) will be automatically calculated in the eCRF as follows:

$$BMI\ (kg/m^2) = Weight(kg)\ \ / Height(m)^2$$

$$TBSA\ (m^2) = 0.007184 \times Weight(kg)^{0.425} \times\ Height(cm)^{0.725}$$

7.1.2 Medical history and breast cancer status

Participant medical history, cancer stage (IA-IIIB), histological type (Infiltrating ductal carcinoma, infiltrating lobular carcinoma, Ductal Carcinoma *in Situ* [DCIS], or other), and grade (1-3), ER and PR status, HER2/neu status will be collected in the eCRF at screening visit.

7.1.3 Previous and concomitant treatments

Previous treatment for breast cancer: chemotherapy, endocrine therapy adjuvant/neoadjuvant (Yes/No), type of breast-conserving surgery (lumpectomy with sentinel node biopsy or lumpectomy with axillary clearance), surgical margins will be reported in the eCRF at the screening visit.

Concomitant treatments (including oncologic and non-oncologic treatments, pain killers, homoeopathic treatment and possible non-medical treatments/devices) will be collected at each visit.

7.1.4 Pregnancy test

Pregnancy test for participants of childbearing potential will be performed at screening (V1) and at V10. The pregnancy test should be repeated at V2 if the screening visit is performed > 10 days prior to the administration of the first dose of the study treatment.

7.1.5 Participant radiotherapy for breast cancer

The RT protocol to be followed by the patient during the study will be reported in the eCRF at the screening visit, including RT technique (3D, IMRT/VMAT), laterality, the total prescribed dose and total dose delivered (Gy), number of planned fractions and fractions delivered, and date of first and last fraction.

At each RT session (V2 to V6), the date, the time and the dose delivered (Gy) will be reported in the eCRF. If the session is cancelled or reported, it will be specified in the eCRF, with the reason for cancellation/delay of the session: RT-related AEs (radiodermatitis, oedema, hyperpigmentation, skin infection, need for topical and systemic antibiotics, need for topical steroids, need for analgesics, need for silicone-based dressings), technical break down, patient condition, patient decision, medical decision, other.

7.1.6 Targeted physical examination

The targeted physical examination will include bilateral breast inspection and palpation, plus bilateral axillary and supraclavicular palpation. Wound healing (surgical wounds, biopsy sites, open wounds in the breast or chest) will also be assessed. The results will be collected in the eCRF. Targeted physical examination will be performed at the screening visit (baseline, i.e., V1), V2 and V6 up to last visit (V10-12).

7.1.7 Performance status

The performance status will be evaluated according to the Eastern Cooperative Oncology Group Performance Status (ECOG-PS) scale. This scale is rated from 0 (Fully active, able to carry on all pre-disease performance without restriction) to 5 (Death). The participant ECOG-PS will be collected in the eCRF at the screening visit (baseline, i.e., V1), V2 and V6 up to last visit.

7.1.8 Vital signs

Heart rate:
HR will be measured at rest (after the subject has been sitting for at least 15 minutes), counting the number of pulses for one minute at the radial artery.

Blood pressure:
The systolic and diastolic BP will be measured at rest (after the subject has been sitting for at least 15 minutes) with an inflatable cuff.

HR and BP will be collected twice for each day of the treatment period (Day 1 to 5, V2 to V6) for all patients (safety run-in and main phase)

- Before the participant applies the drug in the dressing room, before irradiation
- After irradiation, when the participant goes back to the dressing room.

All data will be reported in the eCRF. Any clinically significant changes between pre-dosing and post-irradiation will be recorded as an AE.

7.1.9 Clinical laboratory evaluation

Serum calcium levels will be assessed on safety run-in participants and analysed at a local laboratory. Reference ranges will be supplied by the local laboratory and used by the Investigator to assess the data for clinical significance.

Blood samples for serum calcium will be collected at baseline (during visit 1 or 2) and on the last day of treatment (Visit 6, day 5) at pre-determined timepoints: pre-dose and 2.5 h post-dose.

7.1.10 Radiation dermatitis evaluation

Radiation dermatitis (radiation-induced skin toxicities), as defined by the finding of cutaneous inflammatory reaction occurring as a result of exposure to biologically effective levels of ionizing radiation in the treated region, should be evaluated at each visit and scored according to the latest version of the NCI Common Terminology Criteria for Adverse Events (CTCAE). As per version 5.0, the various grades are defined as follows:

- Grade 1: Faint erythema or dry desquamation
- Grade 2: Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate oedema
- Grade 3: Moist desquamation in areas other than skin folds and creases; bleeding induced by minor trauma or abrasion

- Grade 4: Life-threatening consequences; skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site; skin graft indicated
- Grade 5: Death

7.1.11 Pain and pruritis self-evaluation (ePROs)

The intensity of the worst pain and the peak pruritus (itching) during the previous 24 hours will be daily self-assessed by the participant using a 0-10 NRS, 0 meaning no pain/no itching and 10 meaning maximum pain/maximum itching. Daily self-assessments will be reported via a smartphone application.

7.1.12 Dermatology Life Quality Index (DLQI; ePRO)

DLQI is a ten-item questionnaire used to measure the impact of skin disease on the quality of life of an affected person. It is designed for people aged 16 years and above. There are 10 questions covering the following topics: symptoms, embarrassment, shopping and home care, clothes, social and leisure, sport, work or study, close relationships, sex, and treatment. Each question refers to the impact of the skin disease on the patient's life over the previous week. Each question is scored from 0 to 3, giving a possible score range from 0 (meaning no impact of skin disease on quality of life) to 30 (meaning maximum impact on quality of life).

The questionnaire will be completed by the participant at screening (or at V2 if not completed at screening) and at Visits 6 up to 12 via a smartphone application.

7.1.13 Assessments performed at a designated centre only (patients from the safety run-in and the main phase):

7.1.13.1 Skin colour a* value

The evolution of the breast skin surface inflammation will be assessed through repeated measures of the skin colour a* values using a tristimulus colorimeter in accordance with the CIE L*a*b* colour space (CIELAB) colour systems. The a* value (red-green) will be assessed at screening (V1) or at V2, if not completed at screening, and at V6 up to last visit. Values will be retrieved from the colorimeter by the medical study staff.

7.1.13.2 Transepidermal Water Loss (TEWL)

The TEWL is the loss of water that passes from inside a body through the epidermis to the surrounding atmosphere via diffusion and evaporation processes.

The TEWL will be measured at screening (V1) or at V2, if not completed at screening, and at V6 up to last visit, according to the procedure of the centre. Measurements will be performed on 4 test areas within the radiation field of the breast and on a control area of the volar aspect of the opposite (non-irradiated side) forearm. The values (g/[m²h]) will be retrieved from the device by the medical study staff.

7.1.13.3 Clinical photographs of the treatment area

Clinical photographs of the treated region are optional and will apply only to subjects who provide specific written consent. Clinical photographs will be captured by the investigator at screening (V1) or at V2, if not completed at screening, and at V6 up to last visit using standardised photographic methods. Additional details will be provided in the study manual.

7.2 Pharmacokinetic Assessments

Blood samples for PK assessments (most importantly C_{max}) of amifostine thiol will be collected from up to 30 participants during the safety run-in on the last day of treatment (day 5) at pre-determined time points:

- Pre-dose
- Post-irradiation, i.e., 45 min post-dose
- 1.5 h post-dose
- 2.5 h post-dose

Blood samples will be collected at each time point. Refer to Appendix 1 for total blood volumes. The different steps of the blood sample processing will be described in a laboratory manual.

7.3 Study Committees

The study is being closely monitored by an independent Data and Safety Monitoring Board (DSMB) to ensure the safety and well-being of the participants.
The Steering Executive Committee has the responsibility of approving any protocol amendments and ensuring the study's scientific integrity is maintained in accordance with the established protocol. Furthermore, they are committed to overseeing the trial's ethical conduct, reviewing the recommendations made by the DSMB, and taking appropriate actions to guarantee the safety of the participants. The committee also diligently reviews and assesses publications and presentations of the Study Data to ensure accuracy and validity.

A multidisciplinary Quality Assurance committee for RT (RT-QA committee) will be implemented for this trial. The RT-QA committee will ensure consistency in the RT procedures, review deviations, and offer support to investigators. Its composition and responsibilities will be detailed in an RT-QA committee charter.

8 STUDY INTERVENTION

The study will involve the use of two topical formulations:

- **GR1014-CG gel 4.7% and 2.4%** (50 and 25 mg/mL), a topical formulation of 2-[(3-aminopropyl) amino]ethanethiol (amifostine thiol, WR-1065, GR1014) the active metabolite of amifostine (Ethyol®).
- **Vehicle gel**, a topical formulation without amifostine thiol.

8.1 Study Intervention(s) Administered

Table 4 - Medicinal product specifications

Study Intervention Name:	**GR1014-CG**	**Vehicle**
Pharmaceutical form:	Gel (product in liquid form at room temperature that gels when applied onto the skin)	
Unit dose strength(s)/Dosage level(s):	4.7% and 2.4% (50 and 25 mg/mL)	-
Route of Administration:	Cutaneous	
Dosing instructions:	Topical application on the skin surface to be irradiated (at a dosage of 0.5 mL per 100 cm^2)	
Packaging and Labelling:	20 mL clear type I glass vial closed with a grey silicone freeze dryer stopper and a flip tear-up seal clear aluminium white capsule. All investigational products used in this study will be prepared, packaged, and labelled in accordance with the GMP guidelines, ICH guidelines for GCP and applicable regulations.	
Storage Conditions:	Store in a freezer between -25°C and -15°C	

8.2 Investigational Product Dose Regimen

GR1014-CG or vehicle gels are to be applied topically on the skin surface to be irradiated (0.5 mL per 100 cm^2) each day of the RT, 15-30 min before irradiation.

8.3 Investigational Product Packaging and Labelling

All investigational products used in this study will be prepared, packaged, and labelled in accordance with the Sponsor or its representative GMP guidelines, ICH guidelines for GCP and applicable regulations.

8.4 Investigational Product Preparation – Storage – Handling – Administration

IMP will be provided as daily kits. After randomisation, each participant will be attributed a set of 5 daily kits to be kept in a secured, temperature-controlled freezer in the radiotherapy service/hospital pharmacy between -25°C and -15°C. Temperature excursions must be reported to the Sponsor.

Procedure before IMP administration to participant:

- 30 minutes to 1 hour prior to application of the IMP to the participant, take 1 vial out of the freezer to let it thaw and adjust to room temperature.
- Alternatively, take 1 vial out of the freezer in the morning and store it in a temperature-controlled refrigerator (2 to 8°C). Take the vial out of the refrigerator 30 minutes prior to the application of the IMP to the participant to let it adjust to room temperature.
- A vial should not be left at room temperature for more than 4 hours.

Instructions for use and administration:

Detailed instructions will be provided to the sites. Nitrile gloves should be used by the participant when applying the IMP.

Briefly,

- The investigator will determine the amount of gel to be applied for each participant during the first visit (based on a volume of 0.5 mL of gel per 100 cm^2 of skin).
- Each day of the RT, approximately 15 to 30 min before the RT session, the IMP is to be applied by the participant under a health care provider's (e.g., radiotherapist) supervision, generously over the whole zone to be irradiated with a gentle massage until complete gel formation, using disposable nitrile-gloved hands.
- The product is to be left on the skin for at least 15 min.
- The treated skin is to be thereafter gently cleansed using soft watered wipes and carefully patted dry without rubbing, under supervision, before participant RT.

The calculated volume of IMP to be applied at each RT session will be reported in the eCRF at screening.
At each RT visit (V2 to V6), the kit number, the actual volume of IMP applied, the date, the time of application, and the time of cleansing will be collected in the eCRF.

Note: in case of omission of an IMP application, it will be indicated in the eCRF, and no additional doses will be applied (the IMP will not be applied at a double dose the next day, it will not be applied after the RT session).

8.5 Investigational Product Compliance - Accountability

When participants are dosed at the site, they will apply the product (where physically able) under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of trial intervention and trial participant identification will be confirmed at the time of dosing by a member of the trial site staff other than the person administering the trial intervention.

The number of vials for a participant is to be recorded in a tracking form by the investigator or a designated person from his/her team.

8.6 Concomitant Therapies

Any medication or vaccine (including over-the-counter or prescription medicines, recreational drugs, vitamins, and/or herbal supplements) or other specific categories of interest that the participant is receiving at the time of enrolment or receives during the trial must be recorded on the eCRF and participant files along with:

- Reason for use
- Dates of administration, including start and end dates
- Dosage information, including dose and frequency, potential dose adjustments, etc.

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Necessary supportive care, such as antibiotics, antiemetics, antidiarrheals, etc., will be allowed. Over the course of this trial, additional medications may be required to manage aspects of the disease state of the participants, including AEs from administered treatment(s) as per the investigator's judgement.

Care must be taken if participants are using anti-hypertensive medications, as hypotension has been seen with systemic use of the study drug.

8.7 Radiation Therapy

8.7.1 Prescription

Radiation therapy will deliver 26.0 Gy in 5 fractions of 5.2 Gy over 1 week (5 Fr/week) to the whole treated breast. No bolus should be used. No tumour bed boost will be delivered.

8.7.2 Volume definition

Whole Breast Clinical Target Volume (CTVp_breast) includes the soft tissues of the whole breast from 5 mm below the skin surface down to the deep fascia, excluding muscle and underlying rib cage.

Planning Target Volumes (PTVp_breast) is obtained by adding a margin to CTVp_breast, taking into account set-up error, breast swelling and breathing. A typical CTV to PTV margin is 5 mm in all directions. PTV should be cropped to 5 mm beneath the skin. However, the actual setup error depends on the immobilisation of the patient and the image guidance strategy, and, therefore varies among departments. Each department should perform measurements to determine their CTV to PTV margin.

Organs at Risk (OAR): It is mandatory to contour the Contralateral breast (Breast_contra), ipsilateral lung (Lung_ipsilat), contralateral lung (Lung_contra), both lungs (Lungs) and heart (Heart) for dose-volume histogram assessment. The heart should be outlined from the inferior aspect above the diaphragm to the superior aspect below the pulmonary arch. Volumes are recorded for the purposes of the trial.

8.7.3 Participant position

The patient must lie supine in a stable and reproducible position. The same position must remain for simulation, CT scanning and treatment. An immobilisation device, such as a breast board with arm and wrist supports, an arm pole and/or a vac-fix bag, should be used. Ideally, the immobilisation should allow daily reproducibility of ±5 mm. The patient must not be moved during each fraction.

8.7.4 Acquisition of outlines

A full 3D CT covering the whole breast and the organs at risk must be collected with a slice separation of no more than 3 mm. The imaging technology to be used must be x-ray CT only to provide accurate dose-volume histogram (DVH) data for plan assessment.

8.7.5 Radiation therapy planning

It is compulsory to outline target volumes and the relevant organs at risk for RT planning. All computer planning must be carried out on a 3D dataset, and correction for tissue heterogeneity must be applied. Usually, a tangential pair beam arrangement is used to encompass the whole breast PTV, minimizing the contralateral breast, ipsilateral lung and heart in the fields. The treatment plan must be optimised with 3D dose compensation, aiming to fulfil the dosimetric constraints in the study manual. Fixed or rotational IMRT are authorised, provided that dosimetric constraints are respected. Electron beams must not be used. Deep inspiration breath hold for simulation, CT scanning and treatment is recommended. Beam energy for treatment is usually 6 MV, but a mixture of energies, e.g. 6 MV and 15 MV can be used

for larger patients in order to fulfil dosimetric constraints as per the study manual and avoid cold and hot spots.

8.7.6 Treatment scheduling and gaps

Treatment should preferably be delivered from Monday to Friday in 5 consecutive days but can start on any day of the week. A gap of up to 3 days is acceptable in the event of machine service or breakdown. This is preferable to transferring the patient to a machine on which daily verification imaging is unavailable. If the treatment machine is unavailable for more than 3 days, the QA team should be contacted.

8.7.7 Radiation therapy verification

Treatment Set-up verification is carried out using electronic portal imaging of the treatment beam. This can be either MV or kV. This is required for each fraction to check for a gross error. A tolerance of not more than 5 mm should be used. Local policy is followed if the check is out of tolerance. A further image may be taken to confirm the correction, and this also applies where daily imaging is used to correct the couch position before treatment. The best practice is to correct all measured displacements.

8.7.8 *In-vivo* dosimetry

All patients should have *in-vivo* dosimetry within the first fractions of treatment. This may be performed using diodes or thermo-luminescent dosimetry (TLD). Other methods may be appropriate for individual centres and should be discussed with the QA team.

8.7.9 Radiation therapy quality assurance

A comprehensive quality assurance programme is planned for all centres involved (See Study Manual).

9 RANDOMISATION AND BLINDING PROCEDURES

9.1 Randomisation

Participants will be randomly assigned through Interactive Web Response System (IWRS) to one of the three treatment groups (4.7% GR1014-CG, 2.4% GR1014-CG, vehicle group) using a block randomisation procedure to allow for the following stratification factors:

- Bra cup size: < D vs ≥ D.
- Previous chemotherapy: yes vs. no.

Excelya will provide two dedicated independent statisticians to create and maintain the randomisation list and the kit list. All statisticians in charge of the creation of the

randomization list(s) with access to the lists (randomization and kits) at any time before release of randomization list(s) need to be independent from clinical trial activities. The randomization statistician and the QC statistician/programmer must maintain strict confidentiality. A restricted access area is set-up with exclusive read and write access for the randomization team. Any seed used for creation of the randomization list(s) as well as the created randomization list(s) itself are kept exclusively in this restricted access area until final release of the randomization list(s).

The Excelya randomisation statisticians will be the point of contact of the IWRS team.

9.2 Blinding procedures

After randomisation, each participant will be attributed a set of 5 daily kits of the treatment (blinded packaged) to be kept in a secured freezer in the radiotherapy service/hospital pharmacy.

Both active investigational treatments (GR1014-CG) and the vehicle have the same visual appearance. Both the active and the vehicle have an odour, although different, that can be detected at close contact and is unlikely to be perceived during clinical application.

In order to maintain double-blind conditions:

- the participant and the supervising health care provider should wear a surgical mask during the application and the cleansing,
- the identity of the treatment (GR1014-CG or vehicle) assigned, or its odour properties will not be communicated to the participant and investigator,
- the nature of the treatment (GR1014-CG or vehicle) assigned will not be communicated to the investigational staff; any staff member who could identify the nature of the treatment should make their best efforts not to communicate it to the participant or any other staff members, especially those evaluating the participant.

9.2.1 Unblinding

The double-blind will be maintained throughout the study until the final database lock for the final analysis for all involved personnel on the sponsor site and at Excelya.

Members of the DSMB (who have to be independent from the personnel involved in the study) are allowed to have access to the unblinded treatment information.

In case of medical or safety reporting emergencies, the unblinding of an individual can be done to reveal the treatment assignment for that specific participant.

If a treatment assignment is unblinded, the patient must discontinue protocol therapy but will continue in the study for all safety assessments.

9.2.2 Emergency Unblinding

Unblinding can be performed in cases of an emergency and is based on the fact that the patient's acute well-being and proper management may benefit from the treating physician's knowledge of the patient's treatment assignment or for conditions in which unblinding would otherwise influence the management of the medical situation/clinical judgement. Examples of emergencies include but are not limited to life-threatening adverse events that are at least possibly related to the investigational agent and for which unblinding would influence treatment decisions.

The investigator has the sole responsibility for determining if unblinding of a patient's treatment assignment is warranted. Patient safety must always be the first consideration in making such a determination. If a patient's treatment assignment is unblinded, the Sponsor should be notified immediately. Emergency unblinding for AEs must be performed through the IWRS. All calls resulting in an unblinding event are recorded and reported by the IWRS.

10 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with applicable regulations, ICH Guidelines and Guidelines for Good Clinical Practice.

10.1 Definitions

10.1.1 Adverse event

An Adverse Event (AE) is any untoward medical occurrence in a participant administered a medicinal product which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether considered related or not to the medicinal product.

In relation to Laboratory/ vital signs, clinically significant changes from baseline and other clinically significant abnormalities should be recorded as AEs.

The following events may be exempt from reporting:

- Any pre-existing disease or condition or laboratory finding that is present prior to the IMP administration and that does not worsen.
- Laboratory abnormalities determined by the investigator to be not clinically significant.

- Expected progression of the disease being studied
- Hospitalisation for elective surgery or social admission.

10.1.2 Adverse drug reaction (ADR)

All noxious and unintended responses to a medicinal product related to any dose should be considered ADRs.

All AEs judged by either the reporting Investigator or the Sponsor as having a causal relationship to a medicinal product qualify as an ADR.

10.1.3 Serious adverse event or reaction (SAE/SAR)

An SAE or SAR is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires in-patient hospitalisation or prolongation of existing hospitalisation.

NOTE: Any hospital admission with at least 1 overnight stay will be considered an inpatient hospitalisation, with the exception of planned trial-related overnight stays. An emergency room visit without hospital admission will not be recorded as an SAE under this criterion, nor will hospitalisation for a procedure planned or scheduled before signing the Informed Consent Form (ICF). However, unexpected complications and/or prolongation of hospitalisation that occur during elective surgery should be recorded as AEs and assessed for seriousness. Admission to the hospital for social or situational reasons (i.e., no place to stay, living too far away to come for hospital visits) will not be considered inpatient hospitalisations.

- Results in persistent or significant disability/incapacity,
- Results in a congenital anomaly/birth defect,
- Is an important medical event, as per the investigator's judgment.

NOTE: Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

10.1.4 Adverse event of special interest (AESI)

An AESI is a pre-specified event that has the potential to be causally associated with the IMP regardless of seriousness and, therefore, needs to be carefully monitored and followed up.

10.1.5 Unexpected adverse event /adverse reaction (UAE/UAR)

An adverse event/reaction, the nature, severity, or outcome of which is not consistent with the applicable Reference Safety Information in the product information (Investigator's Brochure for GR1014-CG). When the UAER is a serious reaction, we call it SUSAR.

10.1.6 Unexpected events/urgent safety measures

All unexpected events that might materially influence the benefit-risk assessment of the medicinal product or that would lead to changes in the administration of a medicinal product or the overall conduct of a clinical trial are notified to the Agencies of the countries concerned. Examples of such unexpected events include an increase in the rate of occurrence of expected serious adverse reactions which may be clinically important, a significant hazard to the patient population, such as lack of efficacy of a medicinal product.

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator shall take appropriate urgent safety measures to protect the participants. The CRO, on behalf of the Sponsor, shall notify the Member States concerned, through the EU portal (CTIS), and MHRA of the event and the measures taken. That notification shall be made without undue delay, but no later than seven days from the date the measures have been taken.

10.1.7 Serious breach

'Serious breach' means a breach likely to affect to a significant degree the safety and rights of a participant or the reliability and robustness of the data generated in the clinical trial. The CRO, on behalf of the Sponsor, shall notify the Member States concerned about a serious breach of this Regulation or of the version of the protocol applicable at the time of the breach through the EU portal (CTIS) and MHRA, without undue delay but not later than seven days of becoming aware of that breach.

10.1.8 Treatment-emergent adverse events (TEAE)

A TEAE is an AE that occurs after IMP dosing or that was present prior to dosing but becomes exacerbated between dosing during a treatment period and the end of the trial.

10.2 Assessment of Adverse Events

The following parameters will be assessed for any adverse event recorded within the trial:

- Intensity/Severity,
- Causality,
- Seriousness,
- Expectedness.

The investigator will be responsible for the initial assessment of Intensity/Severity, Causality and Seriousness. The sponsor will be responsible for the assessment of Intensity/Severity, Causality, Seriousness and Expectedness.

10.2.1 Assessment of intensity/severity

The intensity/severity will be assessed by using the National Cancer Institute's Common Terminology Criteria for AEs (CTCAE, latest version) by both the investigator and the Sponsor:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

10.2.2 Assessment of causality

The causality assessment will be performed by the investigator and Sponsor. The causality assessment performed by the investigator should not be downgraded by the Sponsor. In case of different causality assessments of unexpected serious adverse events, both causalities will be indicated in the report made by the Sponsor to the Competent Authority (CA).

In the eCRF AE form and SAE/AESI form, the causality is evaluated:

- Compared to the trial (act, method, related products),
- Compared to trial products (active/comparative),
- According to the criteria: related (reasonable possibility), not related (no reasonable possibility).

The causality could be considered as not related (no reasonable possibility), only whether:

- A clear alternative explanation exists, and/or,
- The event occurs according to a chronology incompatible with the time of trial product intake and/or with the trial conduct and/or,
- The relationship between the trial and the event is not plausible.

The causality could be considered as related (reasonable possibility) only whether:

- The event occurs according to a chronology compatible with the time of trial product intake and/or with trial conduct and/or,

- The relation between the event and the trial is plausible, but the event could also be explained by concurrent disease or other drugs or chemicals or,
- The event is part of adverse events expected in the context of the trial.

10.2.3 Assessment of seriousness

The assessment of the seriousness of an adverse event is based on the definition mentioned above (section 10.1.3). The decision to qualify an event as serious or not is generally taken by the investigator carrying out the notification. The Sponsor also assesses the seriousness of the adverse event considering the assessment of the investigator. Upgrading by the Sponsor is allowed, and the most upgraded assessment will be considered for reporting to the Competent Authority.

10.2.4 Assessment of the unexpected nature

The assessment of the expectedness of an adverse event is based on the definition mentioned above (section 10.1.5).

10.3 Outcome

The outcome of a reported AE as described at the time of reporting (not inferring a causal relationship) can be any of the following:

- Unknown
- Recovered/Resolved
- Recovering/Resolving
- Recovered with sequelae: The participant has recovered, but symptoms/pathology persist
- Not recovered/Not resolved
- Fatal

10.4 Actions Regarding Study Intervention Administration

The actions relative to study intervention administration in the case of AEs should be documented in the eCRF. The following applies:

- Unknown
- Drug withdrawn
- Dose reduced
- Dose increased
- Dose not changed

10.5 Adverse Event Information

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.

The investigator will then record all relevant AE/SAE information in the eCRF within 24 hours of awareness.

For each AE recorded in the AE section of the eCRF, the investigator should include the following information:

1. The severity grade according to the CTCAE criteria.
2. Its relationship to study intervention – causality assessment (reasonable possibility/no reasonable possibility).
3. Its duration (start and end dates or if ongoing).
4. Whether it constitutes an SAE and/or an AESI. If SAE, seriousness criteria.
5. The outcome of AE.
6. Actions regarding the study drug.

Medication errors and uses outside what is foreseen in the protocol, including misuse, overdose and abuse of the study drug, should also be recorded by the investigator in the AE section of the eCRF (regardless of an AE).

It is not acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the /AE/SAE eCRF page.

There may be instances when copies of medical records for certain cases are requested by the Sponsor or designee, or competent authority. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the Sponsor or designee or competent authority.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

10.6 Recording of Adverse Events, Pregnancy Reports

Any AE should be recorded in the form ''adverse event'' of the eCRF within 24 hours of awareness by the investigator. The collection of AEs will start with the signing of ICF and continue until the end of the trial follow-up period.

In the case of SAE/AESI, the investigator should also complete the "SAE/AESI Form" in the eCRF within 24 hours of awareness.

In case of eCRF dysfunction, the SAE/AESI Form will be completed by the investigator on paper and sent to the Sponsor or Sponsor representative as a scanned document via email: clinicalsafety@excelya.com.

Pregnancy reports will be also sent to the Sponsor or Sponsor representative by the investigator by completing the "Pregnancy Notification Form" and sending it via email no later than within 24 hours of awareness. In case of email dysfunction (receipt

of “undeliverable” message), a backup process (“fax”) for the transfer of the Pregnancy Form to the Sponsor will apply.

All AEs will be followed until resolution or stabilisation, until the event is otherwise explained, or the participant is lost to follow-up or is deceased. Investigators are not obliged to actively seek information on AEs after the end of the trial follow-up period. However, if they become aware of any SAE at any time after a participant has been discharged from the trial, and they consider the event to be reasonably related to the trial treatment or trial participation, they must promptly notify the sponsor. Any new information on a previously recorded AE will be recorded as a follow-up information and will be treated for SAEs and pregnancy reports with the same process and within the same timelines as for the initial reports.

10.7 Reporting of Adverse Events

10.7.1 Regulatory reporting requirements

The Sponsor or its designee will collect and record in the Safety Database reports of SAEs, AESIs and pregnancy reports and their follow-ups from the investigator. The sponsor or its designee will collect, from the notifying investigator, all the information necessary for the evaluation of the cases.

The CRO, on behalf of the Sponsor, will submit every SUSAR that occurred in the trial to EudraVigilance and to any other Regulatory Body (as required locally in the involved countries):

- Without any delay for fatal or life-threatening SUSAR and in any case no later than 7 days after knowledge by the sponsor
- Within 15 days from the time the Sponsor is aware of the event for the SUSAR.

Follow-up information must be transmitted within 8 calendar days for fatal or life-threatening SUSARs. For all other SUSARs, the follow-up information will be submitted within 15 days.

The CRO, on behalf of the Sponsor, will be responsible for preparing and reporting the Development Safety Update Report (Annual Safety report) to EMA (via CTIS) and to any other Regulatory Body (as required locally in the involved countries).

10.7.2 Notification of serious breaches, unexpected events and urgent safety measures

Notification of unexpected events with urgent safety measures will be performed as described in section 10.1.6 above. Notification of serious breaches will be performed as described in section 10.1.7 above.

10.7.3 Notification of safety information by sponsor to investigators

The CRO, on behalf of the sponsor, will inform all the investigators concerned of any data that could have an unfavourable impact on the safety of participants, in particular any relevant information resulting from the analysis of SUSAR or in the event of the occurrence of an unexpected event having an impact on the conduct of the clinical trial, including the suspension of the trial program or decision to amend the protocol for security reasons. The CRO, on behalf of the Sponsor, will submit all SUSARs observed in the trial in an aggregate format and in a periodic manner to the investigators and in accordance with local legislation requirements in the involved countries.

10.7.4 Reporting pregnancy

Pregnancy in a trial participant must be reported to the Sponsor or its representative by the site personnel as described in section 10.6 above. Participants who become pregnant during the trial must be withdrawn from the trial.

Pregnancy in a trial participant is not considered an adverse event. However, any congenital anomalies that would lead to an elective or spontaneous abortion are considered a serious adverse event and must be reported to the Sponsor or its representative immediately by the site becoming aware of the event. The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate, and the information will be forwarded to the sponsor. Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the investigator will be reported to the sponsor. While the investigator is not obligated to actively seek this information from former study participants, he or she may learn of an SAE through spontaneous reporting.

10.8 Follow Up of AE/SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor or designee or competent authorities to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other healthcare professionals.
- If a participant dies during the study or during a recognised follow-up period, the investigator will provide the Sponsor or designee or competent authorities with a copy of any post-mortem findings, including histopathology, if an autopsy has been performed.

- New or updated information will be recorded in the originally completed eCRF. The investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

For all AEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

11 STATISTICAL CONSIDERATIONS

11.1 Power and Sample Size Determination

The results of the "FAST-Forward" phase 3 trial show that, using the 26 Gy in 5 fractions RT regimen, 6% of the evaluable patients reported no radiation dermatitis (grade 0, CTCAE) in the substudy 1 and 2, respectively[4]. In their study, Nugent et al.[6] reported that 17% of the patients who underwent 26 Gy in 5 fractions without a boost displayed no skin toxicity (Grade 0).

Based on these results, it is assumed that for this study, among eligible participants undergoing 26 Gy in 5 fractions RT being treated with the vehicle gel, 10% will experience no radiation dermatitis (Grade 0, CTCAE LV) during the course of the study, versus 30% of participants who will be treated with GR1014-CG, i.e., a difference of 20%.

Using a two-sided Pearson-Chi-Square test, 82 participants per arm will be needed to show this anticipated difference in CTCAE LV grade 0 radiation dermatitis rates of 30% and 10%, with a power of 90% and a type I error rate of 5%.

As this study is a first-in-human, two dosages of GR1014-CG will be investigated in a 3-arm parallel group design (GR1014-CG 4.7% high dose [50 mg/mL], GR1014-CG 2.4% low dose [25 mg/mL], and vehicle gel), leading to a total of 246 participants needed.

Assuming 10% of participants to be non-evaluable (dropouts, major deviations from the protocol, etc.), it is planned to accrue 273 participants in total over 10 months in several (~10) centres.

11.2 Analysis Populations

The safety population is defined as all randomised participants having received at least one application of the study treatment.

The modified Intent-to-Treat (mITT) population is defined as all randomised participants having received at least one application of the study treatment.

The Per-Protocol (PP) population is defined as all mITT participants who received the full radiotherapy course and are without any major protocol deviations. All protocol deviations will be listed and assessed as major or minor prior to unblinding of the treatment code. The details of major protocol deviations will be defined in the statistical analysis plan.

The PK population is defined as all randomised participants having had blood drawn at the defined time points prior to and post receiving the study treatment.

The efficacy analyses will be performed on the mITT and the PP population for supportive analyses according to the randomisation group regardless of the study treatment actually received. Any discrepancies between the analyses will be carefully explored.

The safety analyses will be performed on the safety population according to the study treatment actually received.

The analyses of the PK parameters will be performed on the PK population according to the study treatment actually received.

11.3 Efficacy and Safety Analyses

11.3.1 Background and demographic characteristics

All demographic and baseline characteristics, as well as the information on medical history and previous and concomitant medication, will be summarised descriptively for each treatment group, the pooled active treatment groups and overall.

That is, qualitative variables will be described using the absolute number and percentage of each category and the 95% confidence interval (CI) when appropriate.

Quantitative variables will be expressed by the mean, the standard deviation (95% CI of the mean when appropriate), the median, the quartiles (Q1, Q3) and the range (minimum, maximum).

The number of missing values will be given.

11.3.2 Efficacy analyses

Primary endpoint

A summary table will present the percentage of participants with no radiation dermatitis (CTCAE LV grade of 0) for each treatment group, the pooled active treatment groups and overall.

The rate of participants with no radiation dermatitis (CTCAE LV grade of 0) in the higher dosage active treatment group will be compared with the rate in the vehicle group using a Mantel-Haenszel Chi-Square test (considering the stratification factors

bra cup size and previous chemotherapy), and two-sided 95% CIs for the respective rates as well as a two-sided 95% CI for the difference in these rates will be calculated.

Based on the assumptions made for the sample size calculations, to observe a rate of 10% of participants with radiation dermatitis Grade 0 (CTCAE LV) in the vehicle-treated group but 30% in the actively treated group, i.e. a difference of +20%, the hypotheses that will be tested can be formulated as:

H0 (Null Hypothesis): The difference in the rates of participants with no radiation dermatitis in the active treatment group compared to the vehicle group is < 20%.

H1 (Alternative): The difference in the rates of participants with no radiation dermatitis in the active treatment group compared to the vehicle group is ≥ 20%.

The null hypothesis will be rejected if the 95% CI for the difference of the rates lies above the 20% threshold.

Upon rejection of the tested null hypothesis, the rates of participants with no radiation dermatitis (grade 0) in the lower dosage active treatment group will be compared with the rate in the vehicle group with the same methodology, providing two-sided 95% CIs for the respective rates as well as for the difference of them.

Due to the hierarchical structure, no adjustment of the type I error rate will be considered, and both comparisons will be based on a significance level of 5%.

Supportive analyses of the primary endpoint

These analyses (including the hierarchical structure) will also be repeated applying rules for replacement of missing data of the Grade to assess the robustness of the main results. Two sensitivity analyses will be performed:

- Worst case analysis: for participants with at least one missing data of the Grade between Visit 7 and Visit 10, the missing data will be replaced by the event “radiation dermatitis (Grade > 0)”.
- Best case analysis: for participants with at least one missing data of the Grade and no grade > 0 between Visit 7 and Visit 10, the missing data will be replaced by the event “no radiation dermatitis (Grade = 0)”.

The handling of missing data will be detailed in the SAP.

Secondary endpoints

A summary table will present the percentage of participants with radiation dermatitis (CTCAE LV grades 1, 2, 3, 4 or 5, respectively) for each treatment group, the pooled active treatment groups and overall.

The same methodology of a Mantel-Haenszel Chi-Square test and two-sided 95% CIs, as well as the hierarchical strategy used for primary efficacy endpoint will be used to analyse the key secondary endpoints of the percentage of participants with radiation

dermatitis grade ≥2 (CTCAE LV) the endpoint of the percentage of participants with a severe radiation dermatitis (CTCAE LV grade ≥ 3), and the endpoint of the percentage of participants with no radiation dermatitis (CTCAE LV, Grade 0), 4 weeks after the last RT session (Visit 10). In the same way as the primary endpoint analysis, the same supportive analyses will be applied for the key secondary endpoints analyses and will be detailed in the SAP.

For all other secondary endpoint analyses no hierarchical strategy will be applied. The endpoints duration of radiation dermatitis grade ≥ 2 (CTCAE LV) and the time to onset of such will be analysed using survival analysis methodology.

Repeated measures analyses will be conducted on the assessed secondary endpoints of radiation dermatitis grades, skin colour values, pain score, pruritus score, TEWL and DLQI response variables, using treatment arm and time point of assessment as classification variables with interaction effects.

The maximum grade of radiation dermatitis reached in each participant as well as the number of cases of side effects of the RT other than radiation dermatitis (oedema; hyperpigmentation; skin infection; need for topical and systemic antibiotics; need for analgesics) at any time between the first RT and up to 8 weeks after the last one will be analysed descriptively.

If possible (i.e., if sample size allows), all analyses will also be executed considering the following additional factors either as covariates or in subgroup analyses:

- BMI < 25.1 vs ≥ 25.1 (expressed in kg/m^2)
- Postmenopausal vs premenopausal
- Current tobacco use (chewing, smoking) (yes vs. no)
- Diabetes (yes vs. no).

11.3.3 Safety analyses

All safety and tolerability endpoints will be analysed descriptively for each treatment group, the pooled active treatment groups and overall.

Generally, all AEs will be summarised by frequency and proportion of total participants from the safety population, by System Organ Classes (SOC) and preferred terms (PT), after coding based on the latest available version of the Medical Dictionary for Regulatory Activities (MedDRA).

The severity of each AE will be graded according to CTCAE LV.

The number of study withdrawals and the number of stops or delays of RT sessions due to safety events other than the side effects of the RT are also considered secondary endpoints and will be presented in the safety population.

Serum calcium levels will be summarised descriptively and displayed for each assessment timepoint and as change from baseline for participants in the safety population.

11.4 Other Statistical Considerations

All statistical analyses will be performed using Statistical Analysis System (SAS®) Viya.

A Statistical Analysis Plan will be created by Excelya and approved by GRAEGIS as a separate document prior to database lock. This document will provide more details on the planned analyses.

The comparison of the results of the lower dose group versus the vehicle group will only be made after the rejection of the null hypothesis between the higher dose group versus the vehicle group, thus implying a hierarchical structure. With this approach, no adjustment of the type I error rate will be considered, and both comparisons will be based on a significance level of 5%.

Analysis of the PK parameters:

All listed PK parameters will be calculated separately for both active treatment groups using a non-compartment model to characterise the concentration-time curve of GR1014-CG with respect to the distribution and elimination of the product.

AUC_{last} and C_{max} will also be evaluated dose normalised, by dividing the respective parameter by the individual dose given.

Analysis of the exploratory endpoints:

Since the data for the two exploratory endpoints, TEWL and Colorimeter measurements will only be collected in a designated investigational centre, the analyses of these exploratory endpoints will be done in subsets of the mITT and PP populations, i.e., participants with available baseline and at least one post-baseline information for the respective endpoint of interest.

11.5 Sources of Bias And Study Limitations

The study may encounter potential biases and limitations, such as:

- **Difficulty Distinguishing Skin Reactions:** It might be challenging to differentiate skin reactions caused by the product from those resulting from radiation.
- **Unblinding Bias with Vehicle:** To mitigate unblinding bias when using a vehicle, participants and healthcare providers can be instructed to wear a good-fitting face mask.

- **Colorimeter Limitations:** Colorimeters have inherent limitations when used in darker skin tones.
- **Limitations in Transepidermal Water Loss Assessment:** The assessment of TEWL may have limitations depending on the methodology used.
- **Recall Bias in PROs:** Patient-reported outcomes may be subjected to recall bias. However, the pain and pruritus score assessment within a 24-hour timeframe and weekly/biweekly completion of the DLQI can help minimise this bias.
- **Selection Bias Based on ECOG and Criteria:** Selection bias may be present due to the firm selection criteria, including ECOG performance status. Such criteria are implemented to ensure participants' safety in an interventional trial.
- **Participant Compliance Bias:** There is a possibility of participant compliance bias, where some participants may not fully adhere to the prescribed treatment regimen, impacting the study results. This is minimised by having the participants apply the product on-site under supervision directly before irradiation.
- **Observer Bias:** The study's outcomes, especially subjective assessments of skin reactions, may be influenced by observer bias, which is mitigated by blinded randomisation.
- **Confounding Variables:** There could be unaccounted confounding variables that might influence the study outcomes, making it challenging to establish a cause-and-effect relationship.
- **Duration of Follow-up:** The study follow-up period (up to 8 weeks) might not be sufficient to capture long-term safety effects. However, acute side effects such as radiation dermatitis typically appear within 1 to 4 weeks of starting radiation and may persist or worsen during the first week after treatment and typically improve and heal about 2 to 4 weeks after completing RT. Therefore, an up to 8-week follow-up in total after completing RT should be long enough to capture the peak of acute toxicity and toxicity healing.
- **Loss to Follow-up:** Vehicle-treated participants are more prone to loss to follow-up since they are more likely to have sub-optimal prevention of radiation-induced local skin reactions.

11.6 Risk/benefit Assessment

11.6.1 Potential benefits

GR1014-CG has the potential to decrease the frequency or severity of radiation-induced dermatitis, enhancing the tolerability of RT and potentially increasing its effectiveness.

11.6.2 Potential risks

The use of GR1014-CG does not necessitate any modifications to the planned course of RT. At the tested doses, there is no observed risk of compromising the antitumour effectiveness of radiotherapy. The IMP may induce local inflammatory reactions, but

they are temporary and resolve on their own. There is a potential risk of skin damage resulting from GR1014-CG, which could exacerbate radiation-induced skin damage. Additionally, there is a risk of immune-mediated adverse cutaneous reactions. However, at the exposure levels studied, the likelihood of experiencing hypotension, hypocalcaemia, nausea, and vomiting appears to be extremely low.

11.6.3 Risk mitigation

The doses under evaluation are deliberately kept at levels that will not lead to substantial local adverse effects. The intention is to limit the extent of irritation to mild or moderate manifestations, including erythema, oedema, pruritus, pain, and mild hyperpigmentation.

By employing such low exposures, the likelihood of any systemic adverse effects is effectively mitigated, ensuring the treatment remains safe and well-tolerated. Measures are in place to detect potential adverse reactions, for example, vital signs are taken pre- and post-dosing.

11.7 Data Safety Monitoring Board Support

An independent Data Safety and Monitoring Board (DSMB) will review accumulated individual safety data. The data to be reviewed will be unblinded.

Pre-specified early stopping rules for either a possible halt of the complete trial or one of the active treatment arms are detailed in the corresponding DSMB Charter and specified in the protocol.

The first DSMB meeting, other than the orientation meeting, will occur when around 30 participants have been randomised and treated as part of the safety run-in phase of this study.

The frequency and agenda of DSMB meetings will occur at time points defined in the DSMB Charter. Should any untoward safety issue be observed or any of the stopping rules be invoked, the DSMB will schedule an immediate meeting to review the relevant safety data. All DSMB members will be independent of the Sponsor and study.

Details of the DSMB responsibilities and procedures will be described in the DSMB Charter.

12 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

12.1 Study Monitoring

According to ICH GCP guidelines, the Sponsor of the study is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of data

recorded in the eCRFs. The Sponsor or its designee is responsible for assigning the study monitor(s) to this study. The study monitor's duties are to aid the investigator and the Sponsor in the maintenance of complete, accurate, legible, well-organised, and easily retrievable data. The study monitor will advise the investigator of the regulatory necessity for study-related monitoring, audits, competent authorities review, and inspection by providing direct access to the source data/documents. In addition, the study monitor will explain to and interpret for the investigator all regulations applicable to the clinical evaluation of an investigational product as documented in ICH guidelines.

It is the study monitor's responsibility to inspect the eCRFs and source documentation throughout the study to protect the rights of the participants; to verify adherence to the protocol; to verify completeness, accuracy, and consistency of the data; and to confirm adherence to study conduct to any local regulations. Details will be outlined in the clinical monitoring plan.

12.2 Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported or entered in the eCRF that are transcribed from source documents must be consistent with the source documents, or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the trial. Also, current medical records must be available.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Sponsor or designee will perform monitoring to confirm that data entered into the eCRF by authorised site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

12.3 Data Collection and Management

This study will be conducted in compliance with the ICH guidelines and in accordance with the Declaration of Helsinki.

This study will use paper or electronic data collection (techniques to collect data directly from the investigational site using eCRFs). The data will be stored centrally in a fully validated clinical database. The investigator is responsible for ensuring that all

sections of each eCRF are completed promptly and correctly and that entries can be verified against any source data.

Data management will be coordinated by the data managers of the Sponsor, vendor and/or designee in accordance with their standard operating procedures for data management and a formal study data management plan.

Adverse events will be coded with MedDRA. Concomitant medications will be coded using the World Health Organization – Drug Reference List.

Records and documents, including signed ICFs, pertaining to the conduct of this trial must be retained by the investigator for 25 years after trial completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

12.4 Data Protection

- Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that their personal trial-related data will be used by the Sponsor in accordance with local/international data protection law. The level of disclosure must also be explained to the participant, who will be required to give consent for their data to be used as described in the informed consent.
- The participants must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorised personnel appointed by the Sponsor, by appropriate Institutional Review Board/Institutional Ethics Committee (IRB/IEC) members, and by inspectors from regulatory authorities. Personal data related to the Investigator and any investigational staff is subject to compliance with applicable personal data protection and security laws and regulations.
- Investigator agrees to adhere to the principles of medical confidentiality in relation to Clinical Trial Participants. The investigator shall not disclose the identity of Clinical Trial Participants to third parties without prior written consent of the Sponsor.
- The contract between the Sponsor and trial sites specifies responsibilities of the parties related to data protection, including handling of data security breaches and respective communication and cooperation of the parties.
- Information technology systems used to collect, process, and store trial-related data are secured by technical and organisational security measures designed to

protect such data against accidental or unlawful loss, alteration, or unauthorised disclosure or access.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Quality Control

This trial will be conducted in compliance with applicable regulatory requirements.

All participant data relating to the study will be recorded on eCRFs. In order to guarantee the authenticity and the credibility of the data in conformity with good clinical practices, the Sponsor has installed a quality assurance system which includes:

- Trial management.
- Quality control of data at the investigating site by the CRA.
- Possible auditing of investigating centres.

13.2 Data Quality Assurance

- All participant data relating to the trial will be recorded eCRFs unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.
- Guidance on the completion of eCRFs will be provided in the accompanying manual/document.
- The investigator must permit trial-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source documents.
- Monitoring details describing strategy, including the definition of trial critical data items and processes (e.g., risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the corresponding documents.
- The Sponsor or designee is responsible for the data management of this trial, including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., contract research organisations).
- Records and documents, including signed ICFs, pertaining to the conduct of this trial must be retained by the investigator for 25 years after trial completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

14 REGULATORY AND ETHICAL CONSIDERATIONS

This trial will be conducted in accordance with the protocol and up-to-date international and local legislation as well as guidelines. These include the following:

- Consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and CIOMS international ethical guidelines
- Applicable ICH GCP guidelines
- Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the trial is initiated
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the trial design, except for changes necessary to eliminate an immediate hazard to trial participants
- Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation, except for changes necessary to eliminate an immediate hazard to trial participants.

14.1 Informed Consent Process

The investigator will explain the nature of the study to the participant or their legally authorised representative and answer all questions regarding the study. Participants or their legally authorised representatives will be required to sign a statement of informed consent that meets the requirements of the applicable legal requirements. Participants must be re-consented to the most current version of the ICF during their participation in the study. A copy of the ICF must be provided to the participant or the participant's legally authorised representative.

The informed consent document will be approved by the competent authorities as appropriate for each study site.

14.2 Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the trial and for 1 year after completion of the trial.

15 ADMINISTRATIVE PROCEDURES

15.1 Publications of the Clinical Study

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments. The Sponsor will comply with the requirements for the publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support the publication of multicentre studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by a mutual agreement. Authorship will be determined by mutual agreement and in line with the International Committee of Medical Journal Editors' authorship requirements. No participant-identifying information will be disclosed in any kind of publication derived from the results of this clinical study.

15.2 Protocol Amendments and Deviations

The Sponsor may modify the clinical trial protocol at any time for ethical, medical, or scientific reasons. Any amendments will be handled according to applicable local regulations.

The Sponsor does not have to notify non-substantial amendments to the competent authorities or the Ethics Committees. However, non-substantial amendments should be recorded and detailed in subsequent submissions, e.g., in the subsequent notification of a substantial amendment.

No waivers to inclusion/exclusion criteria will be granted; participants need to meet all criteria, exactly as specified to be enrolled. Additionally, prospective deviations from the protocol or investigational plan are not permitted except to protect the life or physical well-being of a participant in an emergency. Deviations that occur unintentionally or are the result of action by the participant must be documented and reported to the competent authorities, if applicable, according to regulations. Further details about the documentation, evaluation, and follow-up of protocol deviations are detailed in this study’s clinical monitoring plan.

15.3 Study and Site Termination/Closure

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or investigator may include but are not limited to:

- For trial termination:
    - Discontinuation of further trial intervention development
- For site termination/replacement:
    - Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines;
    - Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator;
    - Total number of participants included earlier than expected.

If the trial is prematurely terminated or suspended, the Sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organisation(s) used in the trial of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should ensure appropriate therapy and/or follow-up.

16 REFERENCES

1. Drost L, Li N, Vesprini D, et al. Prospective Study of Breast Radiation Dermatitis. Clin Breast Cancer. 2018;18(5):e789-e795. doi:10.1016/j.clbc.2018.03.008
2. Brown PE. Mechanism of action of aminothiol radioprotectors. Nature. 1967;213(5074):363-364. doi:10.1038/213363a0
3. Yuhas JM, Storer JB. Chemoprotection against three modes of radiation death in the mouse. Int J Radiat Biol Relat Stud Phys Chem Med. 1969;15(3):233-237. doi:10.1080/09553006914550411
4. Brunt AM, Wheatley D, Yarnold J, et al. Acute skin toxicity associated with a 1-week schedule of whole breast radiotherapy compared with a standard 3-week regimen delivered in the UK FAST-Forward Trial. Radiother Oncol. 2016;120(1):114-118. doi:10.1016/j.radonc.2016.02.027
5. Brunt AM, Haviland JS, Kirby AM, et al. Five-fraction Radiotherapy for Breast Cancer: FAST-Forward to Implementation. Clin Oncol (R Coll Radiol). 2021;33(7):430-439. doi.org/10.1016/j.clon.2021.04.016
6. Nugent K, Quinlan E, Cleary S, et al. Implementation of 26 Gy in five fractions over 1-week adjuvant radiotherapy for breast cancer: Prospective report of acute skin toxicity and consideration of resource implications. The Breast, 2023,6: 55-61. doi.org/10.1016/j.breast.2022.12.008

17 APPENDICES

Appendix 1: Summary of blood sample volumes

	Volume per timepoint	**Screening**	**Day 5 of treatment**
Serum electrolyte (Calcium)	3 mL	3 mL	6 mL
PK sample	3 mL		12 mL
Total		3 mL	18 mL

Appendix 2: History of changes to the protocol

The original Version 1.0 of the protocol has been amended upon MHRA and ANSM request after initial submission. The amended language is **in bold**.

Amended Section	Amendment	Reason for Change
Document History	**Amended Protocol** ~~This is the initial protocol.~~ As necessary, an updated version of this document **has been prepared to** ~~will~~incorporate all revisions to date, including amendments made at the request of the country's competent authorities, institutional review boards/ethics committees (IRBs/ECs), etc.	NSM and MHRA request for changes
Synopsis - Endpoints 4.2.2 Secondary efficacy endpoints	… **Percentage of participants with no radiation dermatitis (CTCAE LV, grade of 0), 4 weeks after the last RT session (at Visit 10).** … ~~Weekly a~~Assessments of radiodermatitis grade (CTCAE LV) between the first RT session and ~~4 weeks after the last one~~**the last visit.** Number of cases of side effects of RT other than radiodermatitis occurring at any time between the first RT session and ~~4 weeks after~~ the last ~~one~~**visit.** … Absolute change from baseline in weekly **averaged** worst skin pain score in the irradiated area. Worst pain intensity (during the last 24 hours) will be scored daily on a 0-10 Numeric Rating Scale (NRS) from first to last visit. Absolute change from baseline in weekly **averaged** peak pruritus score in the irradiated area. Peak pruritus intensity (during the last 24 hours) will be scored daily on a 0-10 NRS~~,~~ from first to last visit. Total score and sub-scores of Dermatology Life Quality Index (DLQI) ~~weekly~~ addressed~~,~~ from the first RT session ~~and 4 weeks after the last one~~**to the last visit.** … Breast skin surface inflammation evolution will be assessed through a repeated measures analysis of the skin colour a-star (a*) values calculated using a tristimulus colo~~u~~rimetric instrument in accordance with the CIELAB colour systems ~~weekly~~ from the first RT session ~~and 4 weeks after the last one up~~ **to the last** ~~study~~ **visit.** Transepidermal water loss (TEWL) ~~weekly~~ measured from the first RT session ~~and up~~ **to the last** ~~study~~ **visit** ~~4 weeks after the last one,~~ for 4 test areas within the radiation field of the breast (1 site per quadrant) and on a control area of the volar aspect of the forearm on the non-irradiated side. …. **The last visit may be at 4 weeks, 6 weeks or 8 weeks after the last RT session, depending on subject's attainment of radiodermatitis Grade 0 (CTCAE LV).**	Addition of a secondary endpoint, rephrasing of some of the existing secondary endpoints and renumbering of secondary endpoints to comply with request for extension of follow-up from ANSM and MHRA.
Synopsis – Study population Section 6.1 Inclusion Criteria	Inclusion criterion 2 has been updated: Those of childbearing potential must be using ~~>99%~~ **highly** effective contraception methods **during the study and for 30 days**	Inclusion criterion 2 update to comply with ANSM request: The inclusion criterion 2 should mention the use of highly effective contraception

Amended Section	Amendment	Reason for Change
	after the last administration of the study treatment and have a negative pregnancy test at screening **and no more than 10 days prior to administration of the first dose of study treatment**. The following section has been added in section 6.1: **Contraception** **Female subjects must agree to use one or more methods of highly-effective contraception during the course of the trial and for 30 days after the last administration of the trial drug.** **A highly effective birth control method is defined as one which can achieve a failure rate of less than 1% per year when used consistently and correctly. Such highly effective birth control methods include:** • **Hormonal methods of contraception, including combined (estrogen and progestogen containing) hormonal contraception; progestogen-only hormonal contraception associated with inhibition of ovulation; intrauterine device (IUD); intrauterine hormone-releasing system (IUS);** • **Non-hormonal methods of contraception, such as bilateral tubal occlusion and a vasectomized partner (on the understanding that this is the only one partner during the whole trial duration and that the surgical success of the vasectomy has been medically assessed).** **Recommendations regarding methods of contraception may differ depending on cancer type (e.g., status of oestrogen and progesterone receptors).**	methods instead of <99% effective methods. Inclusion criterion 2 updated to comply with MHRA request: -The Sponsor must affirm that the screening pregnancy test is implemented no fewer than 10 days prior to the first dose administration. - The protocol sections that address contraception must be revised to indicate that contraception must be used for 30 days after the last dose of therapy.
Synopsis – Study population 6.2 Exclusion criteria	The following exclusion criteria have been updated: • Pregnant **and breastfeeding** women. 8.Patients suffering from scleroderma, autoimmune disease, microvascular diseases, collagen tissue diseases, lupus, pre-existing loss of skin integrity, active eczema in the region to be treated, or **with a history of any of the following: drug-induced severe cutaneous adverse reaction (SCAR; including, but not limited to Stevens-Johnson syndrome/toxic epidermal necrolysis [SJS/TEN], or drug reaction with eosinophilia and systemic symptoms.**	Exclusion criterion 1 is updated to comply with ANSM request: To clearly exclude pregnant women form the recruitment, the exclusion criterion 1 should mention “pregnant and breastfeeding women”. Exclusion criterion 8 updated to comply with MHRA request: As amifostine is associated with severe cutaneous reactions and this trial is of a topical application of amifostine, patients with a history of any of the following: drug-induced severe cutaneous adverse reaction (SCAR; including, but not limited to Stevens-Johnson syndrome/toxic epidermal necrolysis [SJS/TEN], or drug reaction with eosinophilia and systemic symptoms must be specified as excluded.

Amended Section	Amendment	Reason for Change
Synopsis – Methodology 5.2.1 Study periods Schedule of Assessments Tables 1-3 Figure 2 Study visits	… **The participants will be followed for up to 8 weeks after the last RT session.** Safety run-in (in 3 designated investigational centres):~30 participants to allow for ~8-10 evaluable participants per arm, i.e., all 30 participants will have completed the study with ~~4~~**up to 8** weeks follow-up after the last RT treatment. … The PK assessments **and** serum calcium level measurement ~~and vital signs (heart rate [HR] and blood pressure [BP])~~ will ~~also~~ be collected in this group of participants. … Visit 2 … **Pregnancy test should be repeated if the screening visit is performed >10 days from first dose administration.** … Visit 6 **Participants will receive study treatment or a placebo (vehicle gel) under supervision** … Visits 7 **up to** 1~~2~~**0** (V7 **up to** V1~~2~~**0**) – ~~Weekly~~ ~~f~~Follow-up visits for ~~4~~ **up to 8** weeks after the last RT session … **For Visit 10 only, pregnancy test for participants of childbearing potential.** … **End-of-Study (EOS) visit:** Visit 10 (4 weeks after the last RT session**)** will be the end of study visit **for the participants with radiodermatitis Grade 0 (CTCAE LV).** **If participants have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 10, they will perform an additional visit 6 weeks after the last RT session (Visit 11). The EOS visit will be Visit 11 for the patients with radiodermatitis Grade 0 (CTCAE LV).** **If participants have radiodermatitis higher than Grade 0 (CTCAE LV) at Visit 11, they will perform an additional visit 8 weeks after the last RT session (Visit 12).** **…** <u>Early termination visit:</u> ~~Collection of AEs and concomitant medications~~ **The same assessments and procedures as the one of the EOS visit will be performed.** During the study, from first to last visit, participants will assess pain and pruritus using 0-10 NRS (ePRO)~~,~~ every day. DLQI questionnaire (ePRO) will be completed by the participants at the time of V~~v~~isit~~s~~ 1, **or Visit 2 if not done at Visit 1** (baseline)~~,~~ and Visit 6 **up to Visit** ~~to~~ 1~~2~~**0**.	Updated to comply with ANSM and MHRA request to extend follow-up. Updated to comply with MHRA request: • A second pregnancy test scheduled at Visit 10. The Sponsor must affirm that the screening pregnancy test is implemented no fewer than 10 days prior to first dose administration. • Heart rate and blood pressure measurements will be determined in all participants, not only run-in participants. Clarifications were added and typing errors were amended in the synopsis text per visit to ensure consistency with protocol text and Schedule of assessments tables
Synopsis Statistical Considerations	…Repeated measures analyses will be conducted on the ~~weekly~~ assessed…	Updated to comply with ANSM and MHRA request to extend follow-up.

Amended Section	Amendment	Reason for Change
	…The same methodology (**including the hierarchical structure)** of a Mantel-Haenszel chi-square test and two-sided 95% confidence intervals for the difference of rates will be used to analyse the following secondary endpoints: percentage of participants with grade ≥2 radiation dermatitis (CTCAE LV), percentage of participants with a severe radiodermatitis (CTCAE LV grade ≥ 3), **and percentage of participants with no radiation dermatitis (CTCAE LV, grade of 0), 4 weeks after the last RT session (at Visit 10)…** **The safety population is composed of all participants having received at least one application of the study treatment** **The PK population is composed of all randomised participants having had blood drawn at the defined time points prior to and post receiving the study treatment**	Please see below in row referring to Statistical considerations
3.2 Study rationale	**Up to 8**~~4~~ ~~-~~week**s** follow-up of the participants is planned. ~~as t~~**T**he signs and symptoms of radiodermatitis typically appear within 1 to 4 weeks of starting radiation and may persist or worsen during the first week after treatment. They usually improve and heal about 2 to 4 weeks after completing RT. ~~An up to~~**Eight (8)** ~~8 4-~~ weeks follow-up in total after completing RT should be long enough to capture the peak of acute toxicity and toxicity healing.	Updated to comply with ANSM and MHRA request to extend follow-up.
5.1 Overall study design 5.2 Study visits	…over **approximately** 10 months… …The participant will be treated for 5 days (before each RT session) and will be followed for **up to 8**~~4~~weeks after termination of radiotherapy, leading to a total study duration per participant of **approximately** ~~5 weeks~~ **10 weeks.**	Updated to comply with ANSM and MHRA request to extend follow-up.
6 Study Population	**6.7 STOPPING RULES** **6.7.1 Early Stopping Rules** **The termination of the study or one of the arms with the investigational product during the run-in phase will be considered by the DSMB if 3 or more participants in either arm with GR1014-CG present with adverse events of CTCAE grade 4 that are assessed by the Investigator as possibly related to the investigational product.** **6.7.2 Advancement to the Main Phase** **At the end of the safety-run-in, DSMB can propose that one or both active arm(s) will proceed to the main phase if less than 30% of subjects experience dermatitis Grade ≥3.**	The DSMB charter stopping rules are copied the protocol to comply with the corresponding request from MHRA
7 Study Assessments And Procedures 7.1 Clinical and Paraclinical Assessments	For all assessments in this section: …V6 up to ~~V10~~ **last visit.** **7.1.4 Pregnancy test**	The visits have been updated to comply with ANSM and MHRA request to extend follow-up.

Amended Section	Amendment	Reason for Change
	Pregnancy test for participants of childbearing potential will be performed at screening (V1) and at V10. The pregnancy test should be repeated at V2 if the screening visit is performed > 10 days prior to the administration of the first dose of the study treatment.	Pregnancy test section was added to comply with MHRA request: Protocol Table 2 must be revised to indicate a second pregnancy test scheduled at Visit 10. The Sponsor must affirm that the screening pregnancy test is implemented no fewer than 10 days prior to first dose administration.
7.1.6 Targeted physical examination 7.1.7 Performance status	…will be performed at the screening visit (baseline, i.e. V1), **V2 and V6 up to last visit** ~~and at each visit~~.	Vital signs section was updated to comply with MHRA request: Heart rate and blood pressure measurements will be determined in all participants, not only run-in participants. Updated as a clarification to the point that "Subjects should be carefully monitored during and after GR1014-CG local administration".
7.1.8 Vital signs	~~…Heart rate and blood pressure~~**HR and BP** will be collected ~~on safety run-in participants at the screening visit (Visit 1) and~~ twice each day of the treatment period (Day 1 to 5, **V2 to V6) for all patients (safety run-in and main phase)**…	
7.1.13.1 Skin colour a* value 7.1.13.2 Transepidermal Water Loss (TEWL) 7.1.13.3 Clinical photographs of the treatment area	…at ~~baseline~~screening (V1~~-~~) or **at V2, if not completed at screening**.~~V2),~~ and at V6~~,~~ up to **last visit**~~V10~~…	
8 STUDY INTERVENTION	…-25°C and -1**5**~~0~~°C	Storage conditions updated according to IMPD
Synopsis statistical considerations 9 Randomisation and Blinding Procedures Randomisation	…Participants will be randomly assigned **through Interactive Web Response System (IWRS)**… …**Excelya will provide two dedicated independent statisticians to create and maintain the randomisation list and the kit list. All statisticians in charge of the creation of the randomization list(s) with access to the lists (randomization and kits) at any time before release of randomization list(s) need to be independent from clinical trial activities. The randomization statistician and the QC statistician/programmer must maintain strict confidentiality. A restricted access area is set-up with exclusive read and write access for the randomization team. Any seed used for creation of the randomization list(s) as well as the created randomization list(s) itself are kept exclusively in this restricted access area until final release of the randomization list(s).**	Protocol section 9 has been updated to comply with: MHRA request to specify the method by which an investigator can rapidly determine the allocation of a participant in an emergency. ANSM request that: The sponsor adds to the protocol the measures that will be put in place in order to account for missing data for the evaluation of efficacy endpoints. Randomization procedures must be described more thoroughly in the trial protocol. Indeed, the methods that will be used for the randomization as well as the team that will perform the randomisation must be described in the protocol. It is specified for each population whether the participants will be analysed as randomised or as treated.
9.2.1 Unblinding	**The Excelya randomisation statisticians will be the point of contact of the IWRS team**…	
9.2.2 Emergency unblinding	…**If a treatment assignment is unblinded, the patient must discontinue protocol therapy but will continue in the study for all safety assessments**…	

Amended Section	Amendment	Reason for Change
	Unblinding can be performed in cases of an emergency and is based on the fact that the patient's acute well-being and proper management may benefit from the treating physician's ~~requires~~ knowledge of the patient's treatment assignment or for conditions in which unblinding would otherwise influence the management of the medical situation/clinical judgement. Examples of emergencies include but are not limited to life-threatening adverse events that are at least possibly related to the investigational agent and for which unblinding would influence treatment decisions. **The investigator has the sole responsibility for determining if unblinding of a patient's treatment assignment is warranted. Patient safety must always be the first consideration in making such a determination. If a patient's treatment assignment is unblinded, the Sponsor should be notified immediately. Emergency unblinding for AEs must be performed through the IWRS. All calls resulting in an unblinding event are recorded and reported by the IWRS.**	
11 Statistical Considerations 11.2 Analysis populations 11.3.2 Efficacy analyses	…The efficacy analyses will be performed on the mITT and ~~on~~ the PP population for supportive analyses **according to the randomisation group regardless of the study treatment actually received**. Any discrepancies between the analyses will be carefully explored. The safety analyses will be performed on the safety population **according to the study treatment actually received.** The analyses of the PK parameters will be performed on the PK population **according to the study treatment actually received.** ~~Analyses on the safety/PK population will be performed according to the study treatment actually received.~~ ~~Analyses on mITT/PP will be performed according to the randomisation group regardless of the study treatment actually received.~~ **<u>Supportive analysis of the primary endpoint</u>** **These analyses (including the hierarchical structure) will also be repeated applying rules for replacement**	

Amended Section	Amendment	Reason for Change
	of missing data of the Grade to assess the robustness of the main results. Two sensitivity analyses will be performed: **Worst case analysis: for participants with at least one missing data of the Grade between Visit 7 and Visit 10, the missing data will be replaced by the event "radiation dermatitis (Grade > 0)".** **Best case analysis: for participants with at least one missing data of the Grade and no grade > 0 between Visit 7 and Visit 10, the missing data will be replaced by the event "no radiation dermatitis (Grade = 0)".** **The handling of missing data will be detailed in the SAP.**	
Secondary endpoints	The same methodology of a Mantel-Haenszel Chi-Square test and two-sided 95% Cis, **as well as the hierarchical strategy used for primary efficacy endpoint** will be used to analyse the key secondary endpoints of the percentage of participants with radiation dermatitis grade ≥2 (CTCAE LV), ~~and~~ the endpoint of the percentage of participants with a severe radiation dermatitis (CTCAE LV grade ≥ 3), **and the endpoint of the percentage of participants with no radiation dermatitis (CTCAE LV, Grade 0), 4 weeks after the last RT session (Visit 10).** **In the same way as the primary endpoint analysis, the same supportive analyses will be applied for the key secondary endpoints analyses and will be detailed in the SAP.** **For all other secondary endpoint analyses no hierarchical strategy will be applied**… …The maximum grade of radiation dermatitis reached in each participant **as well as the number of cases of side effects of the RT other than radiation dermatitis (oedema; hyperpigmentation; skin infection; need for topical and systemic antibiotics; need for analgesics) at any time between the first RT and up to 8 weeks after the last one** will be analysed descriptively.	
11.3.3 Safety analyses	… ~~The summaries of the safety secondary endpoints, i.e., serious adverse reactions and side effects of the RT other than radiation dermatitis (oedema; hyperpigmentation; skin infection; need for topical and systemic~~	

Amended Section	Amendment	Reason for Change
	~~antibiotics; need for analgesics), will also be presented in the mITT and PP populations.~~ The number of study withdrawals and the number of stops or delays of RT sessions due to safety events **other than the side effects of the RT** are also considered secondary endpoints and will be presented in the safety, ~~the mITT and the PP~~ population~~s~~.	
11.5 Sources of bias and study limitations	Duration of follow-up: The study's ~~4-week~~ follow-up period (**up to 8 weeks**) might not be ~~optimal~~ **sufficient** to capture long-term **safety** effects ~~limiting the understanding of the product's safety and efficacy in the long run.~~… Therefore, a**n ~~4~~ up to 8**-week follow-up in total after completing RT should be long enough to capture	The duration has been updated to comply with ANSM and MHRA request to extend follow-up.
11.7 Data safety monitoring board	…Pre-specified early stopping rules for either a possible halt of the complete trial or of one of the active treatment arms ~~will be~~ **are** defined in the corresponding DSMB Charter **and specified in the protocol**…	The DSMB charter stopping rules are copied the protocol to comply with the corresponding request from MHRA
Throughout the text	Correction of typing errors, formatting errors and clarifications added to account for potential inconsistencies between the assessments per visit and the schedule of assessments. Replacement of patient by participant where applicable.	-

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