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A Novel CCK_2/Gastrin Receptor-Localizing Radiolabeled Peptide Probe for Personalized Diagnosis and Therapy of Patients with Progressive or Metastatic Medullary Thyroid Carcinoma – GRAN-T-MTC - A Multicenter Phase I Study.

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Short title: A Novel CCK_2/Gastrin-Based Radiotracer in Medullary Thyroid Carcinoma

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Background

Medullary thyroid carcinoma (MTC) is one of the most challenging cancers. Epidemiological studies have shown that during the past 30 years neither a change in stage at diagnosis nor a significant improvement in survival has been achieved [1]. Therefore, new diagnostic and therapeutic strategies are needed for early detection of metastases/disease recurrence and tumor growth control.

MTC is a neuroendocrine neoplasm deriving from thyroid parafollicular C cells. It accounts for nearly 5-10% of thyroid malignancies. The overall prognosis for MTC patients is relatively good. One third of patients present with a locally invasive tumor or clinically apparent spread to the regional lymph nodes (LNs). Distant metastases are present in 13% of patients at initial diagnosis and portend a poor prognosis. Recurrent disease develops in approximately 50% of patients [2]. Using a prior TNM classification system (American Joint Committee on Cancer/Tumor-Node-Metastasis staging system; 7^{th} Edition) 10-year survival rates for stages I, II, III, and IV are 100%, 93%, 71%, and 21%, respectively.

One third of MTC cases result from a germline activating mutation in the proto-oncogene rearranged during transfection (RET) with a strong correlation between RET mutations and their corresponding phenotypes.

Surgery is the only effective therapy in MTC. Undetectable basal serum calcitonin (Ct) level is a strong predictor of complete remission. When Ct is elevated, careful evaluation and localization of postsurgical remnant tissue/early local recurrence or metastases must be performed. Procalcitonin (PCt) is an independent predictor of MTC progression. High PCt/calcitonin ratios correlate with a high risk of progressive disease and shorter progression-free survival. Serum carcinoembryonic antigen is a useful biomarker for MTC, especially in poorly differentiated metastatic cases with lost ability to produce calcitonin. In some MTC

patients with elevated postoperative Ct and short calcitonin doubling time (CtDT), diagnostic and therapeutic options are limited due to unsuccessful disease localization using established imaging techniques, until basal calcitonin is at least 150 pg/ml [3-5]. Therefore, a search for new targets and corresponding radionuclide imaging biomarkers is warranted.

There is still no efficient, universally recommended treatment regimen for advanced MTC. External beam therapy does not play a significant role. Conventional chemotherapy is of limited value. Newer agents such as irinotecan (a topoisomerase I inhibitor) and 17-AAG (a heat shock protein 90 inhibitor) are being evaluated in clinical trials [6].

Tyrosine kinase inhibitors (TKI) are currently being investigated (Supplementary material, Table S1). Vandetanib (2011) and cabozatinib (2012) have been approved by the US FDA and European Medicines Agency. The low rate of partial responses to TKI therapy and absence of complete responses in any of the various trials of monotherapy emphasize the need for new, more effective agents with acceptable toxicity [7].

In clinical practice, evaluation of patients with MTC engages every available diagnostic option. Anatomic imaging (ultrasound/CT/MRI) and numerous molecular imaging methods (PET/SPECT; Supplementary material, Table S2) have been used to localize recurrence/metastases of MTC. However, there are still many patients with negative imaging results.

The cholecystokinin 2 (CCK_2) receptor is overexpressed in over 90% of MTC cases [8]. Some of the CCK_2 receptor related peptides were tested in clinical pilot studies in humans. Receptor targeting was achieved to some extent in CCK_2/gastrin receptor expressing tissues and, most importantly, in tumor tissue with a high tumor-to-background ratio. The CCK-2/gastrin receptors are also a promising target for peptide receptor radionuclide therapy (PRRT) as the peptide can be labeled with either a γ- or β-emitting radionuclide such as ^{90}Y and ^{177}Lu.

In an attempt to produce therapeutic options with CCK_2/gastrin receptor-binding radiolabeled analogues further research was initiated and coordinated within the European COST Action BM0607 "Targeted Radionuclide Therapy" [9,10]. In these comparative studies, one derivative, namely DOTA-DGlu-DGlu-DGlu-DGlu-DGlu-DGlu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (CP04), showed the most promising characteristics: high metabolic stability and receptor affinity with a high and prolonged tumor uptake against low renal retention and was therefore selected for clinical evaluation [11]. This derivative became a core substance for a new project.

The GRAN-T-MTC consortium operates within the ERA-NET on Translational Cancer Research (TRANSCAN) First Joint Transnational Call for Proposals 2011 (JTC 2011) on: "Validation of biomarkers for personalized cancer medicine". It aims to develop a modern method of advanced MTC treatment, utilizing the phenomenon of overexpression of CCK_2 receptor in this carcinoma.

The project is a phase I study with the ^{111}In-labelled gastrin analogue (DOTA-(DGlu)6-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (CP04). The positive results of the preclinical part of the project confirmed the possibility of administration of ^{111}In-CP04 to humans [12,13].

The trial is registered at www.clinicaltrials.gov (ID: NCT03246659) and has been granted EUDRA-CT number: 2015-000805-38.

The project has been approved by the ethics committees in all centers participating in the clinical part of the project (Supplementary material, Table S3).

The primary aim of the study is to evaluate the safety of the intravenous administration of CP04, to assess the biodistribution and dosimetry of the CCK_2/gastrin receptor ligand (^{111}In-CP04) in cancerous and normal tissues of the human body, and to determine critical organs. The main secondary objectives are: to evaluate the ability of CCK_2/gastrin receptor ligand-scintigraphy to detect cancer lesions and to assess the ability of Gelofusine® co-injection.

Work Package 1 – Radiopharmaceutical development

The first step was to establish a clinically useful formulation for the radiolabeled peptide CP04. CP04 was prepared in GMP quality and tests regarding stability, radiolabeling and toxicity were carried out. A ready-to-use ^{111}In-CP04 kit formulation was prepared, and the Investigational Medicinal Product Dossier (**IMPD)** submitted to the National Authorities of the collaborators [12,13].

Work Package 2 – Clinical trial

Material and methods

A sample size of 20-25 recruited patients with progressive/metastatic MTC is considered appropriate for such a study, being a reasonable compromise to address the study's purpose. The main inclusion criteria are: histologically documented MTC, presence of more than one distant or nodal metastases confirmed with either ^{18}F-FDG PET/CT or contrast-enhanced CT/MRI, or CtDT of less than two years if there is no anatomical evidence of disease, and the exclusion criteria: previous external beam radiation therapy within two years, pregnancy/breast-feeding, known hypersensitivity to gastrin analogues or gelofusine (Supplementary material, Table S4).

Two peptide amounts, both radiolabeled with ^{111}In (200 +/- 10% MBq), were administered: a low, 10 μg, amount was used as a safety step in the first applications of CP04. If its safety was assured, 50 μg (an amount also suitable for PRRT) was applied.

The clinical trial consisted of two phases:

Phase 1a: the first four patients were administered with both 10/50 μg of ^{111}In-CP04.

Phase 1b: only the high peptide amount of 50 μg of ^{111}In-CP04 was given to the next enrolled patients who were randomized for arm 1 or 2 without or with co-administration of Gelofusine® (nephroprotective agent).

All baseline tumor-related signs and symptom assessments were performed before the start of the study. All eligible patients received a centrally assigned number. The tracer was injected within 14 days after the patient's inclusion. According to the Study Protocol subsequent tracer administrations were performed at least two-week intervals, providing that no SAE was observed.

Dosimetry was planned after each tracer injection including activity measurements in blood and urine, and a series of scintigraphic images: dynamic acquisitions of the abdomen region (0-30 min. post injection (p.i.), planar whole body acquisition (30-60 min., 4, 24 and 48 hours p.i.), abdominal and neck/mediastinum SPECT/CT images (4-5 (optional) and 24 hours p.i.). Blood samples were collected for Ct and PCt measurements.

Eight follow-up visits are planned up to 4 months after the tracer administration to assess the CP04 safety profile according to the Common Terminology Criteria for Adverse Events version 4.0.

The study flowcharts are displayed in Figure 1 and the supplementary material (Table S3).

Statistical analysis is done both on an Intention-To-Treat (ITT) and Per-Protocol (PP) basis.

The clinical trial was conducted according to international standards and the principles of the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations and the Declaration of Helsinki.

Initial results

The completed preclinical part of the study showed that ^{111}In CP04 can be prepared using a simple kit procedure, suitable for clinical use. According to the Study Protocol each of the first

four MTC patients enrolled in the study was injected with both peptide amounts (10 μg and 50 μg) of the gastrin analogue ^{111}In-CP04. These initial examinations revealed that ^{111}In-CP04 was safe after intravenous injection (no SAE were reported) and that the MTC metastases could be detected with high sensitivity. Biodistribution and preliminary dosimetry data also showed ^{111}In-CP04 as a promising radiopharmaceutical for PRRT of advanced MTC cases.

Discussion

The main goal of the study is to translate the preclinical research results into the protocols confirming the usefulness of the peptide targeting CCK-2/gastrin receptors as a diagnostic and potentially also a therapeutic tool. Among several new biomarkers investigated in recent years, the CP04 showed good stability and affinity to CCK-2/gastrin receptor *in vitro*, as well as favorable biodistribution and pharmacokinetic properties *in vivo*. For these reasons it has been selected for the current study. CCK-2/gastrin receptors may become viable targets for radionuclide scintigraphy and PRRT, while nephro- and myelotoxicity will be minimized. In the last two decades the somatostatin receptors were instrumental to implement peptide receptor mediated radionuclide diagnosis and therapy into clinical practice. It can be expected that CCK-2/gastrin receptor imaging will become also a valid diagnostic method for a specific and non-invasive staging and follow-up of patients with MTC.

Conclusion:

The project represents a first step towards establishing a new, more efficient strategy for the diagnosis, early detection and therapy of recurrent/ metastatic MTC.

CCK_2/gastrin receptor may become a new target for radionuclide scintigraphy and PRRT (theranostic approach), with nephro- and myelotoxicity minimized by appropriate measures.

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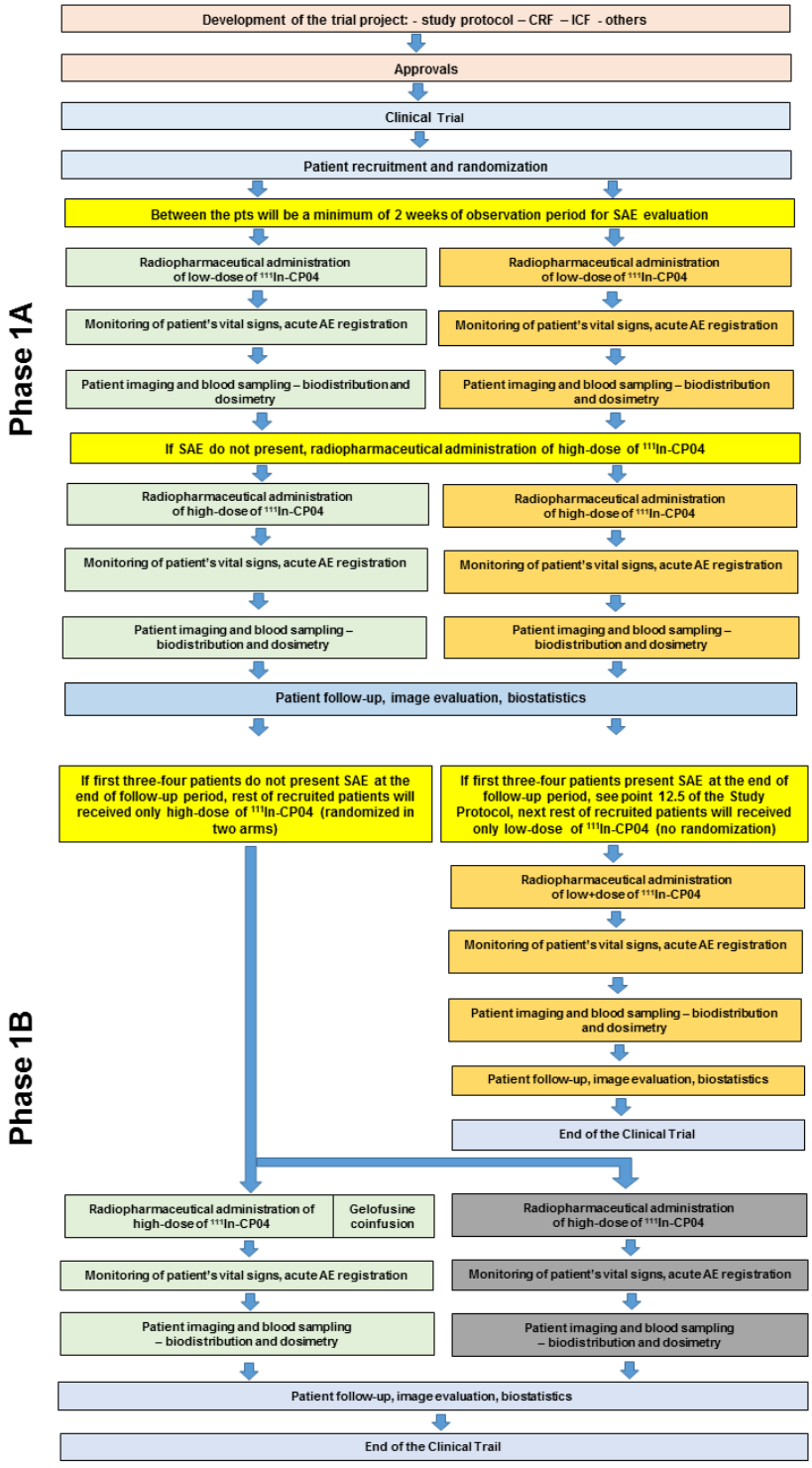

Figure 1. Flowcharts of the Study Protocol.