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Detection of colorectal polyps in humans using an intravenously administered fluorescent peptide targeted against c-Met

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Colon cancer prevention currently relies on colonoscopy using white light to detect and remove polyps, but small and flat polyps are difficult to detect and frequently missed when using this technique. Fluorescence colonoscopy combined with a fluorescent probe specific for a polyp biomarker may improve polyp detection. Here we describe GE-137, a water-soluble probe consisting of a 26–amino acid cyclic peptide that binds the human tyrosine kinase c-Met conjugated to a fluorescent cyanine dye. Intravenous administration of GE-137 leads to its accumulation specifically in c-Met–expressing tumors in mice, and it is safe and well tolerated in humans. Fluorescence colonoscopy in patients receiving intravenous GE-137 enabled visualization of all neoplastic polyps that were visible with white light (38), as well as an additional nine polyps that were not visible with white light. This first-in-human pilot study shows that molecular imaging using an intravenous fluorescent agent specific for c-Met is feasible and safe, and that it may enable the detection of polyps missed by other techniques.

Colorectal cancer (CRC) is a major cause of cancer death[1], and colonoscopy is firmly established as the mainstay of CRC prevention. Evidence that CRC can be prevented by the removal of polyps is strong, especially for cancers of the left colon[2]. However, colonoscopy using current techniques only provides partial protection overall and limited, if any, protection in the right colon[3,4]. Although the aggressive biology of some cancers may in part explain this lack of complete protection, it is likely that limitations in polyp detection using current techniques are primarily responsible[5].

Technical aspects of endoscopic imaging have a major role in determining polyp detection rates, together with human factors such as the quality of bowel preparation and the skill of the endoscopist[6]. Imaging at colonoscopy is currently performed using white light (WL), and polyps are detected by operators who are trained to discriminate polyps from normal colon by recognizing characteristics such as protrusion into the lumen and mucosal color changes. However, these features are less discriminatory in smaller and non-polypoid lesions, leading to miss rates of up to 25% (ref. 7). Combining targeted molecular probes and advanced imaging technology could improve polyp detection. Several biomarkers and detection systems have shown promise in preclinical trials[8,9], but only topically applied agents have thus far been tested in humans[10,11]. These agents suffer from the major disadvantage that application to the whole surface area of the colon is seldom achievable.

c-Met overexpression has been shown to occur as an early event in the colorectal adenoma-carcinoma sequence[12] making it a suitable biomarker for colorectal neoplasia[13]. In addition, its expression on the cell membrane makes extracellular epitopes accessible for targeting with fluorescent imaging agents. Here we report our initial experiences with fluorescence-guided colonoscopy using the imaging agent GE-137 (European Clinical Trials Database registration number 2010-019197-33; publicly accessible via the CCMO register (https://www.toetsingonline.nl/to/ccmo_search.nsf/Searchform?OpenForm). GE-137 is a water-soluble 26–amino acid cyclic peptide labeled with a proprietary cyanine dye ($\lambda_{max\,ex} = 648$ nm) with a high affinity ($K_d = 2$ nM) for human c-Met. We describe the stages of development of GE-137, as well as its safety, pharmacokinetics and imaging characteristics in healthy volunteers and patients at high risk of colorectal neoplasia, in conjunction with a customized colonoscopy imaging system. Because there is currently no commercially available colonoscope with the ability to detect both WL and near-infrared (NIR) fluorescent light, we developed a custom-built dual WL and fluorescent light (FL) endoscopic imaging system specifically for this project.

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It was agreed that the longest wait after injection that was practical for study logistics was ~3 h. The dose of 0.09 mg kg^{-1} showed optimal efficacy at 3 h. It was expected that the colonoscopy exam in patients could take an hour. During this time the contrast agent would continue to wash out. To select the lowest possible dose while ensuring that good contrast was maintained for up to 1 h after the start of colonoscopy, a correction was applied to compensate for the washout. The washout was measured to be $10^{-0.12 \times \text{time}}$ and therefore the following correction was applied:

$$\frac{0.5 \times \text{ECD}}{10^{-0.12}} \approx 0.7 \times \text{ECD}$$

Therefore, an optimal imaging time of 3 h after injection, and an optimal imaging dose of 0.7 times ECD (0.13 mg kg^{-1}) was recommended for use in the pilot study in patients.

Pilot study in patients at high risk of colorectal neoplasia. In 15 patients, a single i.v. dose of 0.13 mg kg^{-1} GE-137 was administered, based on the results of preliminary assessments in the healthy volunteers. Colonoscopy was performed at 3 h after injection. A single experienced endoscopist (J.C.H.H.) performed all the endoscopies. For the patient study the colonoscope was first inserted to the cecum using WL only. The colonoscope was then withdrawn while examining for possible neoplasia with each colonic segment being examined twice. The first-pass examination was performed using WL only. On detecting a lesion with WL, location in the colon, size, visibility in WL and the Paris classification were noted. Lesion visibility was classified as either 'visible lesion' or 'normal flat mucosa'. The lesion was then further examined for visibility in FL and its fluorescence characteristics were noted before biopsy. Fluorescence was related to background fluorescence and categorized as follows: clearly increased, mildly increased or same fluorescence as background. The endoscope was reinserted and a second-pass examination of the same colonic segment performed with combined FL and WL endoscopy. A large WL and smaller FL image were displayed simultaneously on the same monitor. Characteristics of lesions detected on the second pass were noted before biopsy.

Decisions regarding classifications in WL and FL were reached by consensus by the endoscopist and the endoscopy assistant during the procedure. As lesions could be visible in FL based on contours independent of their level of fluorescence, visibility of lesions in FL was assessed after the study day had taken place using the video images that were made during the procedure.

Lesion morphology was described according to the Paris classification and was based on the real-time imaging and snapshot data. Decisions on lesion classification based on review of the images were reached by consensus after assessment by two independent investigators (J.C.H.H. and A.M.J.L.). Size (in mm) was estimated using open biopsy forceps (8 mm) held next to the lesion.

Fluorescence colonoscopy system. A commercial high-resolution video colonoscope was not available to provide simultaneous real-time imaging of WL and GE-137 emission fluorescence. The requirement of highly sensitive detection of fluorescence, as well as full flexibility of imaging parameters, necessitated the development of a custom instrument. To ensure patient safety, the instrument combines a custom image head mounted on the proximal end of a clinically approved lower GI fiber colonoscope (Pentax, FC-38LV) and medical grade components are incorporated where possible. The combined instrument has been tested for safety by an independent medical device testing and certification company (Intertek).

In addition to the commercial colonoscope, the system is comprised of an illumination module and an imaging head (**Supplementary Fig. 6**). The illumination module consists of a 150-W halogen lamp (Pentax LH-150PC) for WL and a high power (3-W) laser diode source (Modulight, ML1496) centered at 635 ± 3 nm coupled into a 600-µm diameter multimode fiber (Thorlabs, M29L01). The laser output power is restricted to Class 3B operation by limiting the light emitted from any accessible apertures to less than 500 mW. The laser drive current is adjusted such that an irradiance of ~10 mW cm^{-2} is achieved at a working distance of 25 mm from the distal tip of the colonoscope.

The WL source and laser are filtered with a short-pass filter at 650 nm (Semrock FF01-650/SP-25) and a laser line excitation filter (Semrock, FF01-628/40-25), respectively, before combining through a dichroic beam splitter (Omega Optical, 615DRSP.) The combined light is mated to the colonoscope illumination port through a custom mechanical adaptor and focused onto the ball lens of the port using an aspheric condenser lens (Thorlabs, AL2520-A).

The imaging head, mounted on the proximal end of the scope, consists of cameras, filters, and imaging optics. Light emerging from the proximal end of the colonoscope passes through a dichroic beamsplitter (Semrock, FF660-Di02-25x36), separating the WL and fluorescence components into separate beam paths. The individual beams are imaged onto independent cameras using a 30-mm achromatic relay lens (Thorlabs, AC254-030-A1-ML) The lens focal length and diameter were chosen to minimize vignetting of the image.

To maximize sensitivity, fluorescence is filtered with an emission filter (Semrock FF01-692/40-25) to filter out wavelengths outside 672–712 nm and detected with a monochrome Peltier-cooled (to −20 °C) electron multiplying charge coupled device (EMCCD, Retriever Technology RT-XMV-247.) The camera pixel count is 658(H) by 496(V), running at eight frames per second (8 f.p.s.) and a 94-ms exposure time. For WL video, a color camera is used (Sentech, STC-CLC152A) with 1392(H) by 1040(V) pixels running at 8 fps and a 40-ms exposure time. To avoid excessive leakage of the red excitation laser into the WL channel, a laser rejection (notch) filter (Semrock, NF03-633E-25) is placed in front of the color camera. The entire imaging head is mounted in a metal housing (Rittal, JB080804HC), which serves as a heat sink for passive cooling of the cameras. The metal housing is attached to the medical cart (Endocart, ED-100B) via an articulated support arm (Bretford, FPSMWADJ2XXAL) that ergonomically supports the weight of the imaging head yet provides six degrees of freedom for the required range of motion.

Images are acquired via a single-board, dual-channel frame grabber with CameraLink interface (Matrox Imaging, Solios eCL SOL-6M-CL-E), using custom image-acquisition software programmed in Microsoft Visual Studio 2005 and Matrox Imaging Library (MIL) version 9.0. The computer system consists of a workstation (Hewlett Packard, xw8600) running 32-bit Windows XP and two 24-inch monitors (Hewlett Packard, LP2475w) one of which is mounted on a support arm (Endocart, EDS-305) and the other on a satellite medical cart (Endocart, EMS-104).

The raw video streams are directly recorded to RAID0 drives in AVI file format. During live image acquisition, video streams from both channels are digitally filtered with a finite impulse response (FIR) filter to remove the fiber honeycomb pattern. FIR kernel sizes up to 9 × 9 pixels may be implemented without appreciable delays on display and recording frame rates. Following each procedure, data is archived to a 1 TB external drive (Western Digital WDE1UBK10000N). Electrical isolation of the entire imaging system is achieved with a medical-grade isolation transformer (Tripp Lite IS1800HG) with up to an 1800-W capacity (**Supplementary Fig. 6**).

Histopathology. All biopsies were formalin fixed, paraffin embedded, sectioned, and stained with H&E according to the standard protocol of Leiden University Medical Center. Final histological evaluation was performed by a single specialist GI pathologist (H.M.). For further analysis all diagnoses were grouped into three basic categories according to the WHO classification of tumors of the digestive system: normal, hyperplastic polyps and adenomatous polyps.

Immunohistochemical analysis of c-Met expression. 4-µm sections were deparaffinized, rehydrated and heat treated (100 °C) in Dako Cytomation Retrieval Buffer pH 9.0 (Dako, Glostrup, Denmark) for 12 min using the Biogenex microwave EZ Retriever (Biogenex, Fremont, CA). Slides were then immersed in 3% hydrogen peroxide solution for 10 min, blocked with Serum-Free Protein Block for 20 min and then incubated for 60 min with a primary rabbit monoclonal antibody specific for the N terminus of human c-Met (antibody EP1454Y, Abcam, Cambridge, UK) at room temperature at a concentration of 1:150 using the i6000 Infinity automated staining system (Biogenex, Fremont, CA). Slides were subsequently incubated with HRP-conjugated goat anti-rabbit IgG for 30 min at room temperature, antibody binding was visualized with diaminobenzidine (Dako) and slides were