

# Recombinant human erythropoietin coupled to near-infrared fluorescence dye Cy5.5

Epo-Cy5.5

Arvind Chopra, PhD<sup>1</sup>

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<b>Chemical name:</b>	Recombinant human erythropoietin coupled to near-infrared fluorescence dye Cy5.5	
<b>Abbreviated name:</b>	Epo-Cy5.5	
<b>Synonym:</b>		
<b>Agent Category:</b>	Protein (glycoprotein)	
<b>Target:</b>	Erythropoietin receptor (Epo-R)	
<b>Target Category:</b>	Receptor	
<b>Method of detection:</b>	Optical imaging (fluorescence-mediated tomography (FMT))	
<b>Source of signal / contrast:</b>	Cy5.5	
<b>Activation:</b>	No	
<b>Studies:</b>	<ul style="list-style-type: none"><li>• <i>In vitro</i></li><li>• Rodents</li></ul>	Structure not available in <a href="#">PubChem</a> .

## Background

[[PubMed](#)]

Erythropoietin (Epo) is a heavily glycosylated chemokine that is expressed primarily in the adult kidney (during the fetal stage of development, Epo is produced mainly in the liver and not the kidneys), mediates its effects through the erythropoietin receptor (Epo-R), and is known to regulate the process of erythropoiesis (red blood cell formation from hematopoietic cells in the bone marrow) in the hematopoietic system (1). Epo-R is also found in other tissues and is believed to protect cells in the cardiac and neuronal tissues

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<sup>1</sup> National Center for Biotechnology Information, NLM, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

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from hypoxic injury and apoptosis. The expression of Epo and Epo-R and the cell signaling pathways involved in bringing about the biological effects of this receptor-ligand complex have been described by Chateauvieux et al. (1). The use of recombinant human Epo (rhuEpo) has been approved by the United States Food and Drug Administration for the [treatment of anemia](#) in certain HIV patients and individuals undergoing chemotherapy for non-myeloid cancer or those who are experiencing chronic kidney failure. Meta-analysis of data obtained from clinical trials has shown that the use of rhuEpo in the clinic is associated with increased adverse thrombo-vascular events or neoplastic tumor progression that results in the mortality of individuals with certain types of cancer (2). In a mouse model of breast cancer it has been shown that, when rhuEpo was used in combination with chemotherapy, there was an increased incidence of cancer metastasis in the animals (3). The co-expression of Epo and Epo-R has been observed in certain neoplasms, including non-small cell lung cancer (NSCLC), and a clinical investigation has shown that expression of these genes in the NSCLC tumors correlated with a negative outcome for the patient (4). However, other studies could not establish a clear link between the expression of Epo and Epo-R and the development of cancer because the Epo-R status of a tumor cannot be determined accurately with currently used immunohistochemical methods, due to the lack of antibodies that can specifically and selectively bind to the Epo-R (5). Therefore, the development of alternative probes that specifically target the Epo-R and can be used with noninvasive imaging techniques to detect and quantify the receptor *in vivo* is necessary (5). Doleschel et al. coupled Cy5.5, a near-infrared dye, to rhuEpo (Epo-Cy5.5) and evaluated the fluorescent conjugate to visualize the expression of EpoR with fluorescence-mediated tomography (FMT) in human lung cancer xenografts in mice (5). The biodistribution of the probe in these animals was also investigated.

## Related Resource Links

[Clinical trials](#) related to erythropoietin

[Nucleotide and protein](#) sequences of human erythropoietin receptor

Erythropoietin receptor in [Online Mendelian Inheritance in Man \(OMIM\) Database](#)

[Human hematopoietic cell lineage pathways](#) (from Kyoto Encyclopedia of Genes and Genomes)

[Anemia](#) in kidney disease and dialysis

Information about [non-small cell lung cancer](#) (from National Cancer Institute website)

## Synthesis

[PubMed]

A commercially available kit of Cy5.5 was used to couple the dye to the free carbonyl groups of the periodate-oxidized carbohydrate side chains of rhuEpo (5). Information

regarding the source of rhuEpo used to produce Epo-Cy5.5, the purification, purity, and yield of the final fluorescent product, and the number of Cy5.5 molecules attached to each molecule of rhuEpo was not reported.

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Epo-Cy5.5 and rhuEpo were reported to generate a similar dose-dependent proliferation curve with UT-7 cells (the proliferation of this human acute myeloid leukemia cell line is Epo dependent), indicating that the biological activity of the Cy5.5 conjugate was the same as the parent molecule (5).

NSCLC cell lines [A549](#), [H838](#), and [H2030](#) were used to determine the *in vitro* binding specificity of Epo-Cy5.5 (5). Before performing the binding study, the expression of Epo-R mRNA and protein in these cell lines was confirmed with quantitative RT-PCR and quantitative immunoblotting, respectively (5). The expression of Epo-R was reported to be higher in the H838 cells (~2000 Epo-R protein/cell) compared with the A549 cells (~250 Epo-R protein/cell), and the H2030 cells did not express any Epo-R. The NSCLC cell lines were exposed to Epo-Cy5.5, and fluorescence microscopy of the different cells at the same exposure times revealed that the fluorescence signal generated by the H838 cells was higher than that generated by the A549 cells, and no signal was observed with the H2030 cells. This indicated that Epo-Cy5.5 had a high binding specificity for Epo-R on these cells.

To further confirm the Epo-R binding specificity of Epo-Cy5.5, Epo-R/enhanced green fluorescence protein (EGFP)-transfected [HeLa](#) cells (Epo-R/EGFP is a fusion protein that has EGFP fused to the C-terminal of the Epo-R; these cells overexpress the fusion protein) were exposed to Epo-Cy5.5 (10 nM) in the absence or presence of excess (190 nM) unlabeled rhuEpo (5). Fluorescence microscopy of the two groups of cells showed that cells incubated with Epo-Cy5.5 alone had a significantly higher ( $P < 0.05$ ) signal intensity compared with cells incubated with Epo-Cy5.5 in the presence of unlabeled rhuEpo. These observations again indicated that Epo-Cy5.5 had a high specificity of binding to the Epo-R (5).

In another study, cryosections of A549 and H838 cell xenograft tumors in nude mice (see below for details) were exposed to Epo-Cy5.5 and examined under a fluorescent microscope (5). Muscle sections obtained from the same animals were used as controls because this tissue does not express Epo-R. A fluorescence signal was detected only in the tumor sections, and the H838 tumor cells generated a stronger signal compared to cells from the A549 lesions. From these observations, the investigators concluded that the level of fluorescence signal generated by the tumor cells correlated with the degree of Epo-R expression in these cells.

## Animal Studies

### Rodents

[PubMed]

To determine the *in vivo* specificity of Epo-Cy5.5, nude mice bearing xenograft tumors generated with either A549 or H838 cells were injected with the conjugate ( $n = 4$  animals/group per cell line) through the tail vein as described by Doleschel et al. (5). Control animals were injected with Epo-Cy5.5 alone (10  $\mu$ M), and the competition group received a 1:5 mixture of Epo-Cy5.5/rhuEpo (10  $\mu$ M:40  $\mu$ M). Micro-computed tomographic (micro-CT)/FMT images were acquired simultaneously from the anesthetized mice at 0.5, 3, 5, 7, 23, 30, 48, and 50 h postinjection (p.i.), and the concentration of Epo-Cy5.5 in tissues of interest was determined by using appropriate software. In general, the tumors of both cell lines in animals injected with Epo-Cy5.5 alone showed a higher concentration of the conjugate at all the time points compared with tumors in mice of the competition group. At 5 h and 7 h p.i., the A549 tumors of control animals had a significantly higher concentration of Epo-Cy5.5 compared with tumors of the competition group ( $57.4 \pm 18.3$  nM *versus*  $21.68 \pm 8.67$  nM;  $P < 0.05$ ), and a similar difference was apparent at 25 h p.i. The H838 tumors in the control animals showed a significantly higher concentration ( $P < 0.001$ ) of the fluorescent conjugate from 7 h to 48 h p.i. compared with the competition group ( $93.96 \pm 11.39$  nM and  $32.44 \pm 7.64$  nM, respectively). These observations indicated that the Epo-Cy5.5 probe bound specifically to the Epo-R in these tumors. Micro-CT/FMT fusion images of the H838 control mice acquired at 50 h p.i. showed a higher accumulation of the probe compared with the competition animals, but the bone marrow from animals of both groups had a similar concentration of Epo-Cy5.5. The investigators observed that the amount of Cy5.5-conjugated Epo in the H838 and the A549 cell tumors correlated with the level of Epo-R expression in these cells.

The biodistribution of Epo-Cy5.5 was investigated in mice bearing A549 cell tumors (5). The animals were injected with the probe as described above, and micro-CT/FMT images were acquired from the animals up to 50 h p.i. The tracer was present in the blood at high levels immediately after injection, and the levels decreased gradually during the next 3 h p.i., followed by a relatively constant level for up to 50 h p.i. Between 3 h and 7 h p.i., the maximum accumulation of the fluorescent conjugate was observed in the liver followed by the kidneys ( $416.77 \pm 135.28$  nM and  $377.75 \pm 227.14$  nM, respectively); subsequently, the kidneys showed a gradual decrease in the levels of Epo-Cy5.5 ( $\sim 100$  nM at 50 h p.i.), whereas in the liver the concentration decreased gradually up to 24 h p.i. and stayed relatively constant ( $\sim 200$  nM) up to 50 h p.i. The amount of Epo-Cy5.5 in the bone marrow stayed almost constant from 3 h to 50 h p.i. (average concentration  $98.24 \pm 8.77$  nM).

From these studies it was concluded that Epo-Cy5.5 can be used to investigate the expression of Epo-R in tumors and the role of this cytokine in the progression of experimental cancerous tumors in mice (5).

## Other Non-Primate Mammals

[PubMed]

No publication is currently available.

## Non-Human Primates

[PubMed]

No publication is currently available.

## Human Studies

[PubMed]

No publication is currently available.

## Supplemental Information

[Disclaimers]

No information is currently available.

## References

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