Reinfusion of Unprocessed, Granulocyte Colony-stimulating Factor-stimulated Whole Blood Allows Dose Escalation of 186Re-labeled Chimeric Monoclonal Antibody U36 Radioimmunotherapy in a Phase I Dose Escalation Study

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ABSTRACT

Purpose: In an earlier Phase I radioimmunotherapy (RIT) study with rhenium-186-labeled chimeric monoclonal antibody (cMAb) U36 in patients with refractory head and neck squamous cell carcinoma, the maximum tolerated activity was established at 1.0 GBq/m², at which bone marrow doses ranged from 0.7 to 1.1 Gy. In the present study, further dose escalation in RIT was evaluated using a facile method of reinfusion of granulocyte colony-stimulating factor (G-CSF)-stimulated unprocessed whole blood.

Experimental Design: Nine patients with recurrent or metastatic head and neck squamous cell carcinoma, the maximum tolerated activity was established at 1.0 GBq/m², at which bone marrow doses ranged from 0.7 to 1.1 Gy. The mean bio-

Results: Blood harvesting, RIT, and reinfusion of whole blood were well tolerated by all patients. G-CSF stimulation resulted in a mean of 0.41 × 10⁶ CD34⁺ cells/kg (range, 0.15–0.83 × 10⁶ CD34⁺ cells/kg) and a mean committed colony-forming units granulocyte macrophage count of 5.62 × 10⁷/kg (range, 0.62–13.37 × 10⁷/kg). The mean biologi-

INTRODUCTION

Radiolabeled MAbs can be used for selective delivery of radiation to tumor sites. For selective treatment of HNSCC, RIT might be an interesting approach as an adjuvant therapy because there is still a high failure rate, either locally or at distant sites, after locoregional treatment of HNSCC with surgery and/or radiotherapy. In patients with hematological malignancies, RIT has shown efficacy, resulting in improved remission and response rates (1, 2). In most of the RIT trials conducted thus far, radiogenic damage to the bone marrow has been the dose-limiting toxicity, resulting in thrombocytopenia and granulocytopenia occurring with a nadir of 4–6 weeks after RIT. Autologous transplantation of bone marrow or separated growth factor-mobilized blood stem cells can reduce myelotoxicity and allowed dose escalation of RIT (3–5). Stem cell transplantation in patients with relapsed B-cell lymphomas treated with high-dose RIT allowed bone marrow-absorbed doses as high as 6.4 Gy before cardio-

Both transplantation of bone marrow and filtered blood stem cells require equipment and laboratory facilities for separation and cryopreservation, whereas both techniques are laborious and expensive. In comparison, G-CSF-stimulated unprocessed whole blood might be an alternative source of blood stem cells. Reinfusion of G-CSF-stimulated whole blood is a straightforward and safe procedure, and it can be performed in a routine clinical setting at low cost (7, 8). The G-CSF-stimulated whole blood can be stored for at least 72 h at 4°C without significant loss of viability of the blood stem cells (9, 10). Whereas up to 90% of the CD34⁺ population shows early apoptotic changes after cryopreservation (11), only 5–10% apoptotic changes were

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The abbreviations used are: MAb, monoclonal antibody; G-CSF, granulocyte colony-stimulating factor; RIT, radioimmunotherapy; cMAb, chimeric MAb; HNSCC, head and neck squamous cell carcinoma; MTA, maximum tolerated activity; CFU-GM, colony-forming unit(s) granulocyte macrophage.
found in the CD34+ population in whole blood transplants after 72 h of storage (12). In multicyclic chemotherapy regimens of short duration, this procedure allowed dose intensification in patients with small cell lung cancer (7, 13). Moreover, myeloablative chemotherapy with whole blood rescue for patients with high-risk lymphoma and multiple myeloma was proven to be feasible (8, 14, 15). To our knowledge, this whole blood procedure has never been applied in RIT studies.

Because the time interval between RIT and the development of myelotoxicity is 4–6 weeks, reinfusion of G-CSF-stimulated whole blood is a realistic option to reduce myelotoxicity of RIT because it allows the reinfused blood stem cells to home and proliferate in the bone marrow before myelotoxicity becomes manifest. A prerequisite for this approach is that most of the radioactivity has disappeared from blood and bone marrow at the time of reinfusion. In an earlier Phase I study, rhenium-186-labeled cMAb U36 was evaluated in patients with refractory HNSCC (16). The MTA (at which a grade 4 hematologic or grade 3 nonhematologic toxicity developed in not more than one of six patients) was found to be 1.0 GBq/m2, with a maximum tolerated bone marrow dose of 0.9 ± 0.2 Gy. Dose-limiting grade 4 myelotoxicity occurred in two of three patients treated with 1.5 GBq/m2. The effective half-life of 186Re-labeled cMAb U36 in blood was 37 h, meaning that after 72 h, 25% of the injected radioactivity was still present in the blood. This effective half-life is related to both the biological half-life of the MAb and the physical half-life of the radionuclide, according to the equation $1/T_{1/2}^{\text{eff}} = 1/T_{1/2}^{\text{bio}} + 1/T_{1/2}^{\text{phys}}$. In the current study, we evaluated whether reinfusion of G-CSF-stimulated unprocessed whole blood after 72 h allows dose escalation of 186Re-labeled cMAb U36 RIT.

### MATERIALS AND METHODS

Nine patients (five men and four women; age range, 48–71 years) were included in this study. Their characteristics are listed in Table 1. All patients had clinical evidence of relapsed HNSCC, either locoregionally or at distant sites, for which no curative options were available. A histologically confirmed HNSCC in the past was required for inclusion. Other eligibility criteria were described in an earlier report on a Phase I dose-escalation study without blood stem cell support (16). The study was approved by the Institutional Review Board of the Vrije Universiteit Medical Center (Amsterdam, the Netherlands). All patients gave written informed consent after thorough explanation of the study.

The antigen recognized by MAb U36 (Centocor B.V., Leiden, the Netherlands) is the keratinocyte-specific CD44 splice variant epitopic. The epitope is located in the v6 domain of CD44 (17). CD44v6 is expressed by squamous cell carcinomas of the head and neck, lung, skin, esophagus, and cervix and also by adenocarcinomas of the breast, colon, lung, and stomach (18, 19). Antibody production, radiolabeling, and quality controls have been described in detail (16). The radiochemical purity of 186Re-labeled cMAb U36 ranged from 95% to 97% as assessed by TLC of the final product. The immunoreactive fraction of 186Re-labeled cMAb U36 ranged from 89.0% to 93.8%.

To mobilize blood stem cells, G-CSF (Filgrastim, Neupogen; Amgen, Thousand Oaks, CA) at a dosage of 10 μg/kg for 5 days was administered s.c. at home. On day 6, just before RIT, two phlebotomies of 500 ml each were performed via an antecubital vein within 2–3 h. Patients were carefully monitored for blood pressure and heart rate during the procedure, and infusion of 500 ml of 0.65% saline was performed after each phlebotomy. Blood was collected in two 2,3-ethyl, hexyl-phtalate plasticized transfer bags (Nederlands Productie Laboratorium voor Bloedtransfusieapparatuur en Infusievoeloestoffen BV, Emmen-Compascuum, the Netherlands) containing 70 ml of CPD as anticoagulant. After collection, the bags were sealed and stored unprocessed and unshaken at 4°C in a temperature-controlled refrigerator. Reinfusion of the unprocessed whole blood was performed after 72 h. The number of cells expressing the CD34 antigen and the committed CFU-GM in stored whole blood were assessed from a sample taken just before reinfusion (10).

Just after collection of G-CSF-stimulated whole blood, 50 mg of 186Re-labeled cMAb U36 were administered i.v. in 5 min. Blood samples were collected from the opposite antecubital vein and counted in a multwell gamma counter (1470 Wizard; 1860 Wizard; 1200Gamma; Packard, Meriden, CT). Radioactivity was determined by TLC of the final product. The immunoreactive fraction of 186Re-labeled cMAb U36 ranged from 95% to 97% as assessed by TLC of the final product. The immunoreactive fraction of 186Re-labeled cMAb U36 ranged from 89.0% to 93.8%.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs/sex)</th>
<th>Disease</th>
<th>Prior treatment</th>
<th>WBC count (× 10$^9$/liter)</th>
<th>CD34$^+$ cells (× 10$^5$/kg)</th>
<th>CFU-GM (× 10$^3$/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60/F</td>
<td>Neck recurrence, lung, and liver metastases of laryngeal carcinoma</td>
<td>SURG$^*$/XRT</td>
<td>35.6</td>
<td>0.28</td>
<td>6.33</td>
</tr>
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<td>2</td>
<td>48/M</td>
<td>Bone metastases of pharyngeal carcinoma</td>
<td>SURG/XRT</td>
<td>44.4</td>
<td>0.17</td>
<td>0.62</td>
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<td>3</td>
<td>58/M</td>
<td>Local recurrence, lung metastases of piriform sinus carcinoma</td>
<td>CHEM/XRT</td>
<td>44.5</td>
<td>0.30</td>
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<td>4</td>
<td>58/M</td>
<td>Neck recurrence, lung and liver metastases of supraglottic laryngeal carcinoma</td>
<td>SURG/XRT</td>
<td>36.6</td>
<td>0.15</td>
<td>3.28</td>
</tr>
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<td>5</td>
<td>54/F</td>
<td>Neck recurrence, lung metastases of tonsillar carcinoma</td>
<td>SURG/XRT</td>
<td>26.6</td>
<td>0.26</td>
<td>6.74</td>
</tr>
<tr>
<td>6</td>
<td>57/M</td>
<td>Neck recurrence, lung metastases after neck lymph node metastases of unknown primary tumor</td>
<td>SURG/XRT</td>
<td>71.6</td>
<td>0.68</td>
<td>2.35</td>
</tr>
<tr>
<td>7</td>
<td>71/F</td>
<td>Cutaneous metastases after neck lymph node metastases of unknown primary tumor</td>
<td>SURG/XRT</td>
<td>48.8</td>
<td>0.66</td>
<td>13.37</td>
</tr>
<tr>
<td>8</td>
<td>67/F</td>
<td>Local recurrence of tonsillar carcinoma</td>
<td>XRT</td>
<td>38.4</td>
<td>0.38</td>
<td>5.34</td>
</tr>
<tr>
<td>9</td>
<td>64/M</td>
<td>Lung metastases of piriform sinus carcinoma</td>
<td>XRT/SURG</td>
<td>59.2</td>
<td>0.83</td>
<td>10.99</td>
</tr>
</tbody>
</table>

* SURG, surgery; XRT, radiotherapy; CHEM, chemotherapy (cisplatin and 5-FU).

WBC was assessed just before RIT; CD34$^+$ and CFU-GM were assessed in stored whole blood just before reinfusion.
Wallac, Turku, Finland) for pharmacokinetic analyses and patient-specific bone marrow dosimetry according to Shen et al. (20). This method takes the contribution of other organs and the whole body into account. The dose levels were 1.0, 1.5, and 2.0 GBq/m² body surface area, and three patients were treated at each dose level. Vital functions (blood pressure, pulse rate, breathing rate, and temperature) were assessed before administration and 20, 40, 60, 120, and 240 min after administration. Patients were admitted for 21 h in a special treatment room at the department of nuclear medicine. Thereafter, they stayed in a single room at the otolaryngology/head and neck surgery ward until reinfusion. Scintigraphic imaging studies were performed with a large field-of-view gamma camera (Dual Head Genesys Imaging System; ADAC Laboratories, Milpitas, CA). Whole-body and lateral, anterior, and posterior planar images and single-photon emission computed tomography of the head and neck region were obtained in all patients. Patients were discharged after the reinfusion was completed.

Hematological parameters were obtained at least weekly until recovery of myelotoxicity was observed. The severity of toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (21). Data on bone marrow dosimetry and development of myelotoxicity were compared with those from patients treated in an earlier study of 186 Relabeled cMAb U36 without the use of whole blood reinfusion (16).

RESULTS

G-CSF treatment for blood stem cell mobilization did not cause side effects in any of the patients. The mean WBC count after G-CSF stimulation was 45.1 × 10⁹/liter (range, 26.6–71.6 × 10⁹/liter; Table 1). Whole blood harvesting did not lead to significant hypotension in patients, and administration of 186 Relabeled cMAb U36, as well as reinfusion of whole blood, was well tolerated by all patients, without occurrence of acute side effects. Scintigraphy showed selective targeting of tumor lesions in all patients, and no accumulation at nontumor sites, except for the presence of radioactivity in feces and urine (Fig. 1). The mean number of CD34⁺ cells harvested after G-CSF stimulation was 0.41 × 10⁶ cells/kg body weight (range, 0.15–0.83 × 10⁶ cells/kg body weight), and a mean CFU-GM count of 5.62 × 10³/kg was found (range, 0.62–13.37 × 10³/kg; Table 1). Pharmacokinetic analysis revealed a mean biological half-life of 72.6 ± 16.0 h for 186 Relabeled cMAb U36 in blood. Bone marrow doses ranged from 0.8 to 2.8 Gy, with a mean bone marrow dose of 0.64 ± 0.13 Gy/GBq (Table 2).

The nadir of platelets was observed 4–5 weeks after administration of 186 Relabeled cMAb U36, and the nadir of WBCs and granulocytes was observed after 5 weeks. In none of the patients was a platelet count of <20 × 10⁹/liter observed. For three patients, platelets were <50 × 10⁹/liter for 5–21 days (Table 2). The granulocyte nadir was <1.0 × 10⁹/liter in four patients, lasting for 2, 4, 7, and 11 days, respectively. Three patients required transfusion of packed RBCs for low hemoglobin levels, which was regarded as grade 3 toxicity. In one of these patients (patient 1), the transfusion was performed because of low hemoglobin level shortly after reinfusion of the unprocessed whole blood, which was therefore most likely the result of hemolysis rather than radiation induced. Patient 4 was given three packed RBCs as well as a single transfusion of platelets because of a sudden low hemoglobin level (4.5 mmol/liter) at 4 weeks after RIT, which was attributed to gastrointestinal bleeding caused by concomitant use of corticosteroids and nonsteroidal anti-inflammatory drugs. At the time of the transfusion, there was grade 3 thrombocytopenia, which showed recovery within 5 days. The other patient (patient 6) was given two transfusions of three packed RBCs for hemoglobin levels of 5.2 and 5.5 mmol/liter at 3.5 and 4 weeks after RIT, respectively.
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increase of the MTA from 1.0 GBq/m² to at least 2.0 GBq/m². The study was established at 1.0 GBq/m², with grade 4 myelotoxicity. The MTA in the previous bone marrow doses from 0.9 to 2.0 GBq/m² was observed as compared with patients treated at the highest dose level showed stable disease. In the previous study in which no reinfusion of G-CSF-stimulated unprocessed whole blood was performed. In the current study, patients tolerated higher total injected doses, and higher doses delivered to the bone marrow were tolerated. Stable disease was more frequently observed in the present study. In total, five of nine patients showed stable disease, and all three patients treated at the highest dose level showed stable disease. In the previous study, only 1 of 13 patients showed stable disease. The only

He suffered from cachexia and frequent bleeding as a result of tumor progression.

Overall, myelotoxicity exceeding grade 3 was not observed (Table 2), and nonhematological toxicity consisted of a grade 2 mucositis in one patient (patient 5). Less severe myelotoxicity was observed as compared with patients treated at the highest dose levels in the previous Phase I study without the use of reinfusion of G-CSF-stimulated whole blood (Table 3; Ref. 16). None of the patients who received up to 2.0 GBq/m² developed more than grade 3 myelotoxicity. The MTA in the previous study was established at 1.0 GBq/m², with grade 4 myelotoxicity in two of three patients receiving 1.5 GBq/m². Introduction of this procedure allowed increase of the maximum tolerated bone marrow doses from 0.9 ± 0.2 Gy to 2.5 ± 0.4 Gy and an increase of the MTA from 1.0 GBq/m² to at least 2.0 GBq/m² (Table 3).

Stable disease was observed in five patients for a period of 3–5 months and is still ongoing in one patient (patient 9). No partial or complete responses were seen.

**DISCUSSION**

In this study, reinfusion of G-CSF-stimulated unprocessed whole blood was used for dose intensification of RIT with 186Relabeled cMAb U36. As in chemotherapy, myelotoxicity, in general, is dose-limiting in RIT studies. Whereas after chemotherapy myelotoxicity becomes manifest within a few days, the nadir of platelets and granulocytes after RIT occurs at 4–6 weeks. This might allow blood stem cells, if reinfused at a time point at which an acceptable low radiation level is present in blood, to home and proliferate in the bone marrow before myelotoxicity becomes manifest, thereby reducing the severity of myelotoxicity.

At the current stage of development, reinfusion of G-CSF-stimulated whole blood should be performed not more than 72 h after harvesting to guarantee the viability of the blood stem cells (9, 10). The effective half-life of 186Relabeled cMAb U36 is 37 h (16), which means that at the time of reinfusion, radiation damage to circulating blood stem cells can still occur.

This preliminary Phase I study showed improved potential of RIT with 186Relabeled cMAb U36 in comparison with a previous study in which no reinfusion of G-CSF-stimulated unprocessed whole blood was performed. In the current study, patients tolerated higher total injected doses, and higher doses delivered to the bone marrow were tolerated. Stable disease was more frequently observed in the present study. In total, five of nine patients showed stable disease, and all three patients treated at the highest dose level showed stable disease. In the previous study, only 1 of 13 patients showed stable disease. The only

**Table 2** Dose levels, BM dosimetry, myelotoxicity, and response after 186Relabeled cMAb U36 RIT with reinfusion of G-CSF-stimulated unprocessed whole blood

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Activity level (GBq/m²)</th>
<th>No. of patients</th>
<th>Mean BM dose (Gy)</th>
<th>Maximum hematological toxicity grade</th>
<th>No. of patients</th>
<th>Mean BM dose (Gy)</th>
<th>Maximum hematological toxicity grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>4</td>
<td>0.3 ± 0.1</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>1.0</td>
<td>6</td>
<td>0.9 ± 0.2</td>
<td>3</td>
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<td></td>
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<tr>
<td>3</td>
<td>1.5</td>
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<td>1.1 ± 0.04</td>
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<td></td>
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<td></td>
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<tr>
<td>4</td>
<td>2.0</td>
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<tr>
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<td>4</td>
<td>0.3 ± 0.1</td>
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<tr>
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<td>1.0</td>
<td>6</td>
<td>0.9 ± 0.2</td>
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<td>3</td>
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<td>1.1 ± 0.04</td>
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<td>4</td>
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</table>

* pRBC, packed RBCs.
* PD, progressive disease; SD, stable disease.
* Patient 4 also received a transfusion of platelets.
* Stable disease ongoing.

**Table 3** Comparison of mean BM dose and maximum hematological toxicity grade after RIT with 186Relabeled cMAb U36 in patients treated without reinfusion of G-CSF-stimulated whole blood and patients treated with reinfusion of G-CSF-stimulated whole blood

<table>
<thead>
<tr>
<th>Activity level (GBq/m²)</th>
<th>No. of patients</th>
<th>Mean BM dose (Gy)</th>
<th>Maximum hematological toxicity grade</th>
<th>No. of patients</th>
<th>Mean BM dose (Gy)</th>
<th>Maximum hematological toxicity grade</th>
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<tr>
<td>1.0</td>
<td>6</td>
<td>0.9 ± 0.2</td>
<td>3</td>
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<tr>
<td>1.5</td>
<td>3</td>
<td>1.1 ± 0.04</td>
<td>4</td>
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</table>

* Patients treated without reinfusion of G-CSF-stimulated whole blood (left, previous Phase I trial described in Ref. 16) versus patients treated with reinfusion of G-CSF-stimulated whole blood in the current study (right). The number of patients treated at each dose level is indicated, as well as the mean BM dose per dose level and corresponding SD.
responder had been treated at the highest dose level. The observation of stabilization of disease in patients treated in the current study offers opportunities for further development of RIT in an adjuvant setting because antibody uptake in small-volume tumors is found to be higher than uptake in large tumors (22, 23).

An aspect for further improvement is the storage time of whole blood, which is currently limited to 72 h. To increase the clinical applicability, as for RIT with radioabeled MABs with a relatively long effective half-life in blood, this storage time should be extended. Encouraging results with new storage mediums have shown preservation of clonogenic capacity of blood stem cells in whole blood for at least 7 days (24), which could allow reinfusion at a later, more suitable time point after RIT, at which remaining radiation levels in blood have been further decreased. For leucopheresis or bone marrow transplantation, this problem of storage time does not play a role. However, both procedures require filtration techniques, cryopreservation, and expensive laboratory facilities. Reinfusion of G-CSF-stimulated unprocessed whole blood can be performed in almost every institution, and at considerably lower costs.

In conclusion, this study showed that a doubling of the MTA of 166Relabeled cMAb U36 could be achieved using reinfusion of G-CSF-stimulated unprocessed whole blood. Our results indicated that bone marrow doses up to 2.8 Gy could be reached without development of dose-limiting myelotoxicity or nonhematological toxicity. In five of nine patients with recurrent disease for whom no curative options were available, stabilization of disease was observed.

REFERENCES


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