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Cetuximab is an active treatment of metastatic and chemorefractory thymoma

Giovannella Palmieri [1], Mirella Marino [2], Marco Salvatore [3], Alfredo Budillon [4], Giuseppina Meo[4], Michele Caraglia [4], and Liliana Montella [1]

[1] Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, [2] Department of Pathology, Regina Elena Cancer Institute, Rome, [3] Diagnostic Imaging and Radiotherapy Department, "Federico II" University, Naples, Italy; [4]Experimental Pharmacology Unit, Department of Experimental Oncology, National Institute of Tumours, Naples, Italy

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1. ABSTRACT

Advanced chemorefractory thymic epithelial tumors still represent a challenge in clinical oncology. A rationale-based therapeutic approach targeting a key pathway should represent the ideal solution in a neoplasm that can over-express Epidermal Growth Factor Receptor (EGFR) in the epithelial component. On the basis of these considerations, two patients with metastatic heavily pretreated disease were evaluated for EGFR expression in the primitive tumor, being considered this data as a basis for an anti EGFR treatment with the monoclonal antibody cetuximab which targets EGFR. A strong EGFR expression was revealed by immunohistochemistry in the two cases considered, thus the patients received cetuximab and reported a partial response as assessed by Computed Tomography (CT), Positron Emission Tomography (PET) and fused PET-CT after three months of therapy. Therefore, both patients are still on therapy. This preliminary experience suggests that cetuximab may be a useful therapeutic choice in advanced pre-treated thymic tumors.

2. INTRODUCTION

New approaches are currently investigated for advanced chemorefractory thymic epithelial tumors. Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with intrinsic tyrosine kinase activity. The EGFR family is a group of four structurally similar growth factor receptors with tyrosine-kinase activity (EGFR, HER2/neu, ErbB-3, ErbB-4), which dimerize upon binding with a number of ligands, including EGF (Epidermal Growth Factor) and TGF (Transforming Growth Factor), allowing downstream transduction of mitogenic signals. Activation results in a variety of cellular responses including cell proliferation and differentiation. Tyrosine kinase oncogenes represent an appealing anti-tumor drug target since they play a fundamental role in a variety of cellular responses including cell proliferation and differentiation. In clinical trials, anti-EGFR is showing promise in the treatment of solid tumors expressing EGFR (1-3). Neoplastic thymoma cells express EGFR (4,5). We evaluated the clinical activity of Cetuximab, a chimeric human-mouse monoclonal IgG1 antibody blocking ligand

binding to EGFR, in two cases of advanced chemorefractory thymic epithelial tumors.

3. MATERIALS AND METHODS

3.1. Patient Characteristics and response evaluation

For patient characteristics see Case report 1 and 2 in the Results section. Given the progressing refractory disease, both the patients were assessed for EGFR expression in the primitive tumor, being considered this data as a basis for an anti EGFR treatment. Both the patients were evaluated as concerns clinical status and biochemistry and were imaged by a combined PET-CT.

3.2. Morphological and Immunohistochemical Methods

The tumours were re-evaluated according to the criteria of the WHO classification (1999) of Thymic tumours (6) and of the 2004 classification of thymic tumours (7). The immunohistochemical study was performed by an EGFR pharmDx assay - a qualitative immunohistochemical kit system – identifying EGFR (HER1) protein in EGFR-expressing cells (DakoCytomation, Carpenter, CA) according to the manufacturer's instructions in routinely fixed, paraffin embedded material. Staining of the tumor cell membrane above the background level was considered positive (1+). Staining intensity was graded 1+, 2+ or 3+ according to the manufacturer's instructions. To validate the staining runs, negative control reagent was used in place of the primary antibody according to the instructions.

3.3. Treatment schedule

EGFR expression showed by neoplastic epithelial cells in the paraffin material from both tumours was considered a support for an anti EGFR treatment *in vivo*. The patients provided informed consent and Local Ethical Committee approved the administration of the drug. Both patients received intravenous cetuximab (C225, Erbitux, Merck KgaA, Darmstadt, Germany) over 1 hour at 400 mg/m^2 loading dose followed by the weekly administration of 250 mg/m^2. The first dose of 400 mg/m^2 was given during the course of 2 hours. Subsequent weekly treatments were given at a dose of 250 mg/m^2 during the course of 1 hour. Premedication with an antihistamine was given according to the recommendations. For the initial dose, the infusion period was 120 minutes. For the subsequent weekly maintenance doses, the infusion period was 60 minutes. Patient evaluation was performed monthly through clinical and laboratory routine examination. The patients n.1 and n.2 started the treatment in November and December 2004, respectively. The first PET-CT evaluation was performed after three months of treatment. The patients were maintained on therapy in the absence of disease progression or unacceptable toxicity.

4. RESULTS

4.1. Case report 1

In 1993, a 36-year-old previously healthy woman presented a combined B2/B3 thymoma according to WHO classification of Thymic Epithelial Tumours (1999) (7) staged III by Masaoka (8). After neoadjuvant PEC (platinum, epidoxorubicin, cyclophosphamide) chemotherapy, she underwent surgical resection of the thymic mass and thereafter received further adjuvant chemotherapy with the same scheme and local radiotherapy (30 Gy). In 1998 a paravertebral relapse at level of D12-L1 vertebrae was shown by Magnetic Resonance Imaging (MRI) and was pathologically confirmed as a metastasis of the same thymic tumour. The patient underwent surgical resection of this metastasis and adjuvant radiotherapy (44 Gy). Three years later, the patient relapsed again in the paravertebral region. Moreover, bone, lung and liver metastases were found. A somatostatin analog-based therapy was started because of patient's refusal of conventional chemotherapy, according to previous described patient's inclusion criteria and therapy schedule (9). A slight reduction of paravertebral relapse and stable disease in the other sites was maintained for about two years. Because of liver toxicity the therapy was stopped and the disease progressed. Therefore, the patient started a chemotherapy regimen including platinum, which was stopped soon because of an allergic reaction.

4.2. Case report 2

In 1990, a 34-year old man suffering from myasthenia gravis underwent surgical resection of B3 thymoma according to WHO '99 classification (6) staged III by Masaoka (8) and following adjuvant radiotherapy. Eight years later multiple pleural nodules were shown. The patient was surgically treated and received chemotherapy (platinum and etoposide). In 1999, he underwent surgery again because of a paravertebral relapse of thymoma at level of D8 vertebra causing medullary cord compression and thereafter received radio- and chemotherapy (cyclophosphamide, vincristine and procarbazine). In 2001, a chest Computed Tomography documented a mediastinal cystic mass measuring 4,5 cm at longest diameter. Moreover, pleural solid tissue was visualized in front of middle lung lobe. Positron Emission Tomography (PET) showed uptake in all these sites including the cystic mass. The patient started a somatostatin analog-based therapy and reported disease stabilization until November 2004 when a progressing paravertebral lesion was discovered. Surgery was considered unfeasible because of significant risk of paralysis.

4.3. Morphologic and immunohistochemical revision

Case n. 1 corresponded to a combined B2/B3 thymoma, and case 2 was considered a B3 thymoma according to the criteria of the 1999 (6) and 2004 (7) WHO classifications of Thymic Tumours. For the EGFR staining, in case n.1 slides of the primary tumor and of a synchronous lymph node metastasis were available, for case n. 2 only material from the primary tumor was available.

The two cases considered were both extensively and strongly membranous positive, graded 2+ in the sections considered. Only epithelial cells reacted, thymoma lymphocytes being unreactive. In Figure 1 staining of case n.1 was shown.

4.4. Clinical responses

After three months of therapy, both the patients achieved a partial response as assessed by CT, PET and

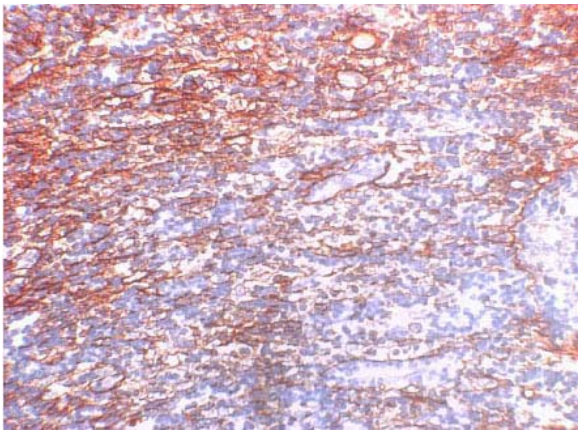

Figure 1. Case 1 Strong membranous positivity for EGFR in B2 thymoma epithelial cells. Lymphocytes are unreactive. 200x

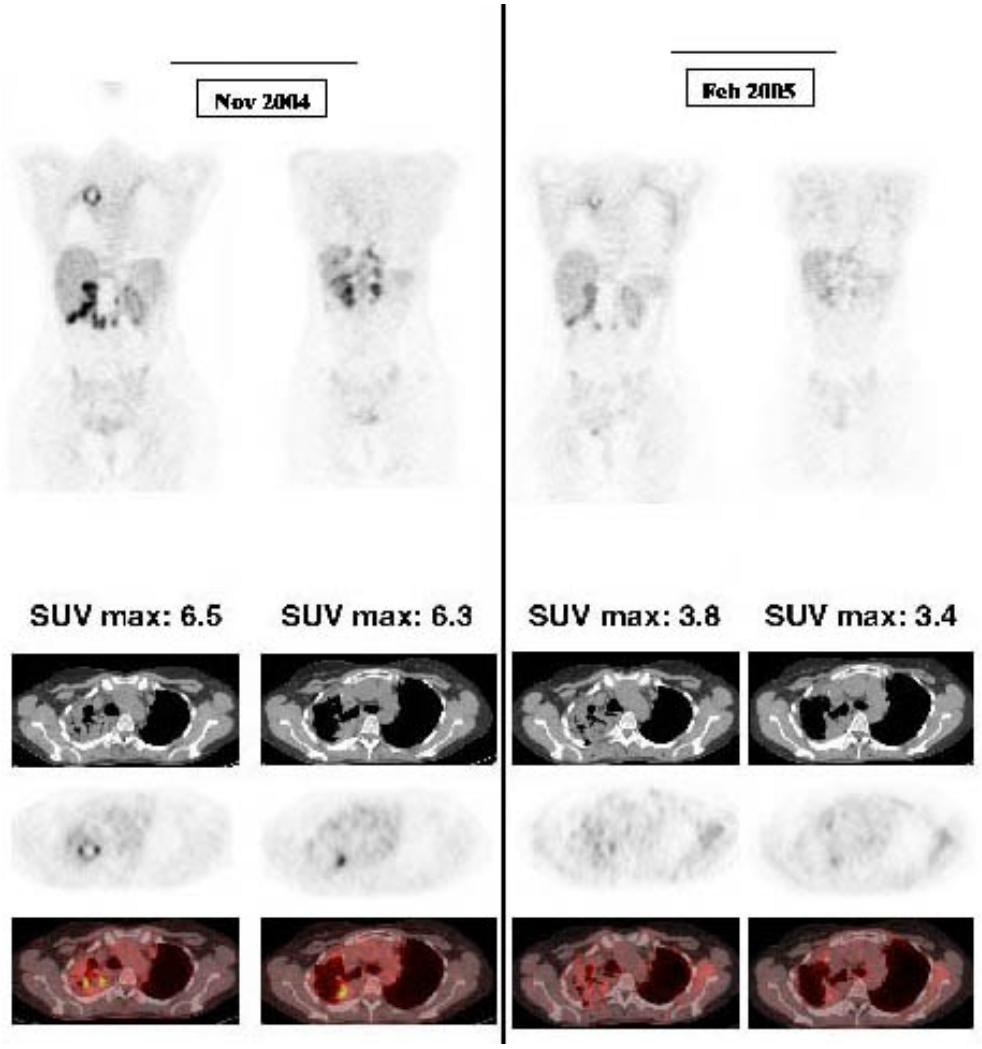

Figure 2. Coronal whole-body [18F]FDG scans of the patient before and after therapy (first row), showing changes of tracer uptake at different metastatic sites. Transaxial slices of CT, PET and fused PET-CT scans at the level of the upper lobe of the right lung (second and third row) showing changes of standardized uptake value (SUV) max before and after therapy.

Table 1. Changes of standardized uptake value (SUV) max in different metastatic sites.

Site	Nov 2004	Feb 2005
Right lung	6.5	3.8
Right paravertebral	7.0	4.2
Left paravertebral	6.6	2.9
Left para-aortic	7.5	4.4
Liver	6	3.0
Sub-costal	5.2	2.6

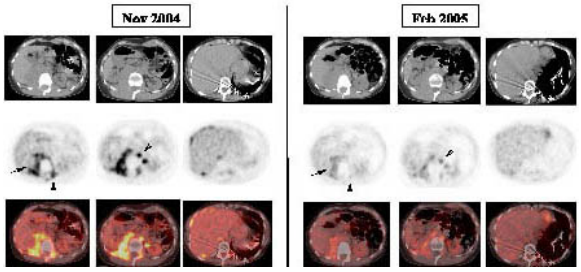

Figure 3. Transaxial slices of CT, PET and fused PET-CT scans before and after therapy showing changes of FDG uptake at the level of the right paravertebral metastasis (arrow), left paravertebral metastasis (arrow head facing upward), para-aortic metastasis (oblique arrow head), liver and sub-costal metastases.

fused PET-CT and therefore are keeping on therapy. The baseline PET-CT study of the patient n.1 showed high [^{18}F]FDG uptake by different metastases including the upper lobe of the right lung, right paravertebral and para-aortic sites (Figures 2 and 3). After therapy, the [^{18}F]FDG uptake significantly decreased at these metastatic sites (Figure 2 and 3 and Table 1) despite apparent lack of changes in tumor size. The only toxicity registered was an acne-like rash in both the patients.

5. DISCUSSION

Clinically evaluated biological treatments of thymic tumors have not shown significant activity until now (10). EGFR was found to be expressed in neoplastic epithelial cells of thymomas, more frequently in metastases and in tissue samples from patients with severe *myasthenia gravis* (4). Increased EGF-R expression may result in increased proliferation of neoplastic cells and also in *myasthenia gravis* (5). Therefore, increased expression of EGF and EGFR may play a role in thymoma progression (11). Very recently, a combined evaluation by immunohistochemistry and fluorescence *in situ* hybridization (FISH) was performed in 32 thymoma specimens, revealing EGFR protein over-expression in 69% of cases and a poor correlation between the protein expression and gene amplification (12). Of 23 specimens with protein over-expression, only 7 (30%) showed EGFR gene amplification by FISH. However, protein expression was retained sufficient in order to antibody administration. In the cases reported here, the EGFR status was investigated by an EGFR pharmDx, a qualitative immunohistochemical kit system evaluating the EGFR (HER1) protein in EGFR-expressing cells. EGFR PharmDx positive testing was considered as an aid in identifying colorectal cancer patients eligible to a treatment with cetuximab.

In this report the response to treatment was assessed also by PET-CT. The role of [^{18}F]FDG PET in restaging after chemo- or radiotherapy in different tumors is well established and PET could be used to detect sub-clinical response even before the appearance of changes in actual tumor size (7,13-16). The reported cases suggest that [^{18}F]FDG PET-CT could be used in metastatic thymoma to monitor the metabolic tumor response to treatment before changes in tumor burden can be appreciated by conventional imaging modalities.

In conclusion, this preliminary evaluation suggests for the first time that cetuximab may be a useful therapeutic choice in advanced pretreated thymic tumors. Despite the need for prolonged follow-up of the patients and further confirmatory studies, the EGF/EGFR pathway seems to play a pivotal role in thymic tumors.

6. ACKNOWLEDGEMENTS

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Send correspondence to: M. Caraglia, MD, PhD, Experimental Pharmacology Unit, Department of Experimental Oncology, National Institute of Tumours of Naples Fondazione “G. Pascale”, Via M. Semmola, 80131 Naples Italy, Tel: 39-081-5903292, Fax: 39-081-5903814, E-mail: Michele.Caraglia@fondazionepascale.it

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