The development of a highly specific mouse monoclonal MET antibody for IHC application

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Abstract

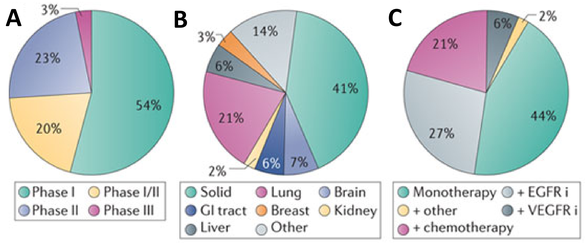
In a wide variety of human solid tumors, the metastasis and poor prognosis are associated with the overexpression of the receptor tyrosine kinase, MET. Several MET inhibitors and antibodies have been tested both in preclinical and clinical trials. Developing companion diagnostic tests to identify the patients who could benefit from these potential medicine is necessary for MET targeted therapies.

IHC tests using formalin-fixed and paraffin embedded (FFPE) tissues are the most commonly used approaches by pathologists. However, the detection of MET protein in FFPE tissues was currently found to be difficult mostly due to the lacking of the specificity and sensitivity of MET antibody. To overcome this limitation, we have developed a specific MET mouse monoclonal antibody (UMB190) by using a high density protein microarray chip and human normal and neoplastic tissues chip technology. Our initial analyses revealed that this monoclonal antibody worked very well in detecting the expression level of MET in FFPE human cancer tissues.

Methods

Antibodies: Mouse monoclonal antibodies were produced by injecting BALB/c mice with recombinant protein fragment corresponding to amino acids 570-885 of human MET produced in E.coli. Protein array: Cell lysates of the trueORF over-expressed in HEK293 were used for printing the protein chip. The array contains a total of 22,176 spots with 10,464 lysates in duplicates (20,928 spots) and 1248 control. Tissues array: The tissue microarray was generated using Sakura's Tissue-Tek Quick-Ray system. On one slide, 12 human normal and 12 human carcinoma tissue samples were spotted as described before [1].

Background



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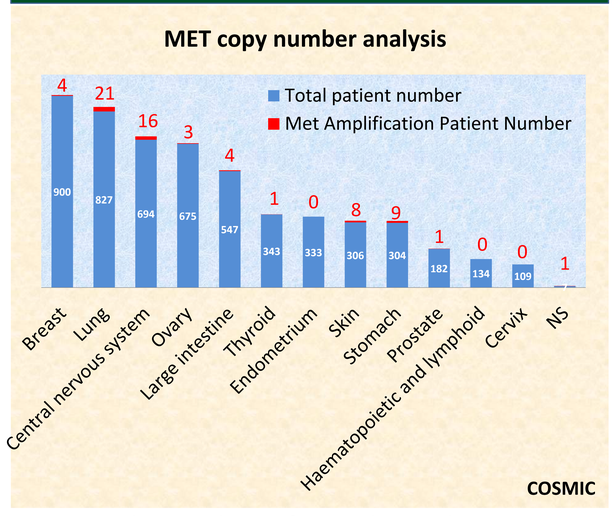
	Patients, n	Median PFS, months	Median OS, months
Erlotinib + Tivantinib	58	4.4	9.9
Erlotinib + Placebo	59	2.3	6.8

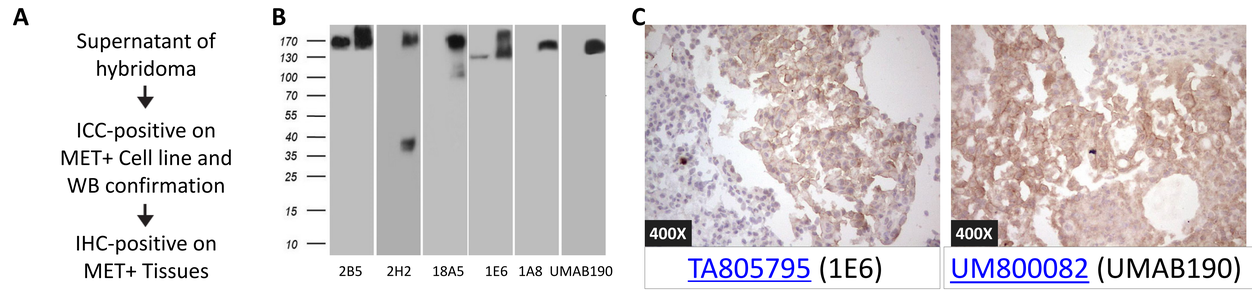
Clinical trials with MET inhibitors. The distribution according to the stage is shown in A, to tumor type shown in B, and to therapeutic strategy shown in C [2]. D, Overall survival in patients with non squamous tumor histology treated with Erlotinib plus Tivantinib or Erlotinib plus Placebo [3].

MET analysis



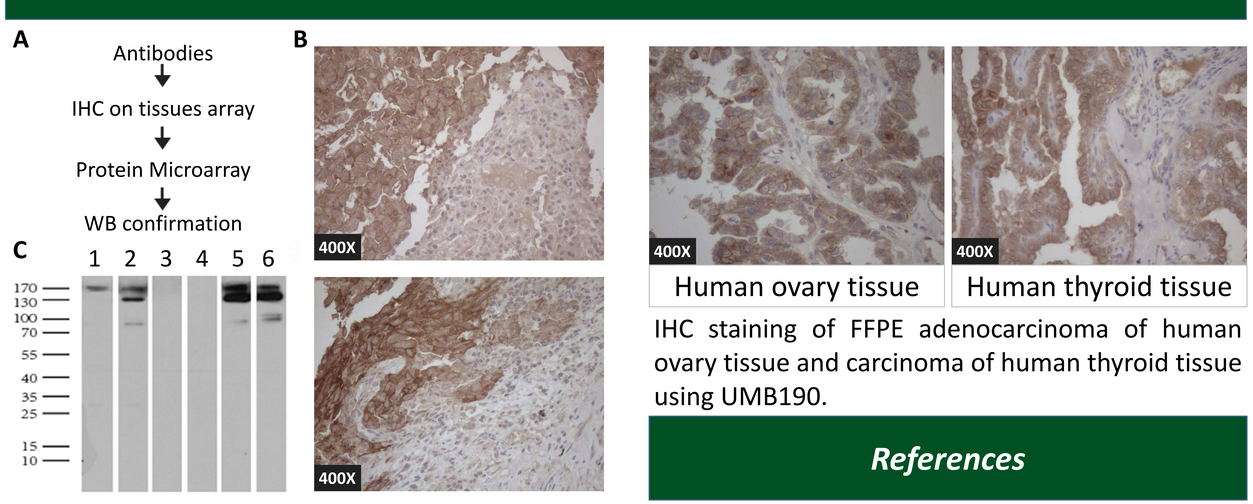
Copy number analysis of MET shows that MET was overexpressed in a wide variety of solid tumors.

Generating MET IHC antibodies



Generating a high-affinity MET antibody for IHC application. A, A hybridoma screening process to select antibodies that recognize MET proteins in formalin-fixed and paraffin embedded (FFPE) tissues. B, Western Blot was used to test the recognition of full length recombinant MET protein (RC217003) by MET antibodies. C, Screening antibodies for IHC application on MET-positive FFPE tissues.

UltraMAB selection process and UMAB190 IHC staining



Selecting MET IHC UltraMAB. A, A UltraMAB screening process to select antibody that have the best specificity. B, IHC staining of FFPE carcinoma of human lung tissue with different MET expression levels using 3G5. C, Western Blot analysis of extracts from 6 different cell lines by using 3G5 (1: MCF7; 2: Hela; 3: Jurkat; 4: PC12; 5: COS7; 6: A549). The 3G5 was selected as UMAB190.

IHC staining of FFPE adenocarcinoma of human ovary tissue and carcinoma of human thyroid tissue using UMB190.

References

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2. Gherardi E, et al. Targeting MET in cancer: rationale and progress. Nature Reviews Cancer, 2012, 12(2): 89-103.
3. Scagliotti G V, et al. Rationale and design of MARQUEE: a phase III, randomized, double-blind study of Tivantinib plus Erlotinib versus placebo plus Erlotinib in previously treated patients with locally advanced or metastatic, non squamous, non–small-cell lung cancer. Clinical lung cancer, 2012, 13(5): 391-395.