Targeting EGFR in bilio-pancreatic and liver carcinoma

Maria Elisabetta Fratto[1], Daniele Santini[1], Bruno Vincenzi[1], Nicola Silvestris[2], Amalia Azzariti[3], Stefania Tommasi[3], Alice Zoccoli[1], Sara Galluzzo[1], Evaristo Maiello[4], Giuseppe Colucci[2], Giuseppe Tonini[1]

[1]Medical Oncology, University Campus Bio-Medico, Rome, [2]Medical and Experimental Oncology Unit, Scientific Institute for Research and Treatment of Cancer "Giovanni Paolo II", Bari,[3]Clinical Experimental Oncology Laboratory, Scientific Institute for Research and Treatment of Cancer "Giovanni Paolo II", [4]Medical Oncology Unit, Scientific Institute for Research and Treatment of Cancer "Casa Sollievo della Sofferenza" – San Giovanni Rotondo

TABLE OF CONTENTS

1. ABSTRACT

The key role of epidermal growth factor receptor (EGFR) in tumorigenesis has been demonstrated in several cancer types, so recent clinical trials have investigated their activity/efficacy in different settings. Two different types of EGFR-targeted agents were developed: monoclonal antibodies such as cetuximab and panitumumab, and tyrosine kinase inhibitors, such as gefitinib and erlotinib. In this review, we summarize the preclinical rational of potential activity and the most important clinical trials evaluated anti-EGFR targeted agents in non-colorectal digestive cancer, both in monotherapy and in combination with other chemotherapeutic or targeted agents. Patient selection by use of biologic markers will identify which patients are more likely to respond, contributing to the successful use of these agents.

2. ANTI-EGFR IN BILIARY TRACT CANCER (BTC)

The overexpression of EGFR and its role in the proliferation of several solid tumors have provided the rationale for targeting this pathway in BTC. Some studies have shown that EGFR is frequently overexpressed in cholangiocarcinoma. Additionally, sustained EGFR activation due to defective receptor internalization has been reported for cholangiocarcinoma cells. EGFR overexpression was shown to be associated with macroscopic tumor type, lymph node metastasis, tumor stage, lymphatic vessel invasion and perineural invasion in extrahepatic cholangiocarcinoma. High levels of EGFR expression and activation has shown to increase the risk for tumor recurrence in intrahepatic cholangiocarcinoma (1). A recent study showed that 15.7% of carcinomas of the gall bladder, 11.5% of ampulloma and 5.1% of extrahepatic bile

duct cancer overexpress ErbB-2 (2). Moreover, a study showed that a subgroup of patients with cholangiocarcinoma or gallbladder carcinoma with somatic mutations of EGFR in the tyrosine kinase domain can elicit cell signals sustaining survival and proliferation (3). Tyrosine Kinase Inhibitors (TKIs) targeting either EGFR or ErbB2, as well as those producing dual inhibition of EGFR and of ErbB2, are able to inhibit cellular growth, inducing apoptosis in human and rodent biliary cancer cell lines *in vitro*. Gefitinib, Lapatinib, Erlotinib and Cetuximab are the most effective agents in pre-clinical studies (4 , 5 , 6). Preclinical evidences of synergistic antitumor activity has emerged combining EGFR inhibitors and rapamycin (7) or Vandetanib, an inhibitor of vascular endothelial growth factor receptor (VEGFR) and EGFR signaling. A recent study showed that vandetanib significantly inhibited the growth of cholangiocarcinoma cells expressing EGFR and VEGF, appearing a promising therapeutic approach for cholangiocarcinoma. The absence of KRAS mutation and the presence of EGFR amplification may be a potential predictive molecular marker of sensitivity to EGFR-targeted therapy in cholangiocarcinoma (8, 9).

Based on the preclinical evidences, many clinical trials are on going to evaluate anti-EGFR therapy in BTC patients. Forty-two patients with BTC were enrolled to receive oral erlotinib (150 mg/die) as monotherapy. 57% of patients had received prior chemotherapy for advanced BTC. HER1/EGFR expression by immunohistochemistry in tumor cells was detected in 29 (81%) of the 36 assessable patients. 7 of the patients (17%) were progression free at 6 months and 3 patients (7%) had partial response. In this study, EGFR mutation status was not tested, so it is unknown if there is a correlation between response and EGFR mutation status (10). This result suggests a benefit of erlotinib in patients with advanced BTC, even if only larger controlled trials and trials evaluating erlotinib will confirm these data.

The role of EGFR inhibitors in BTC was reported in some case reports describing the efficacy of cetuximab in combination with either gemcitabine or gemcitabine and oxaliplatin (11). Cetuximab in combination with gemcitabine and oxaliplatin (GEMOX) in nine GEMOX resistant patients with advanced intrahepatic cholangiocarcinoma was evaluated in a small study. After 6 months, CT scans revealed 1 complete response, 1 partial response, 1 stable disease and 6 patients with disease progression. Median time to tumor progression and overall survival were 4 and 7 months, respectively. So the addiction of cetuximab seemed to reverse the resistance to GEMOX (12 , 13). BINGO trial is a multicenter, randomized phase II trial evaluating the efficacy of GEMOX alone or in combination with biweekly cetuximab in first-line in patients with advanced BTC. The primary end-point was PFS at 4 months. Secondary endpoints were response rate, Progression Free Survival, Overall Survival, toxicity, early response assessment by Positron Emission Tomography (PET) and blood/tumor EGFR signalling pathway member analyses. 101 patients were enrolled from October 2007 to October 2008. At the interim analysis, the 4-month PFS rate was 44% versus 61% in the arm with

cetuximab, so the addiction of cetuximab to GEMOX showed promising activity. This trial is still ongoing. The EGFR pathway analyses will show if there is a correlation between EGFR overexpression and response to cetuximab therapy (14). Recently, a single center Phase II study evaluated the correlation between K-Ras status and response in thirty patients with advanced or metastatic cholangiocarcinoma or gallbladder cancer treated with cetuximab plus GEMOX. The RR was 63,3%, including three patient with a complete response. 5 patients (16,7%) achieved stable disease and only 6 patients (20%) progressed under chemotherapy. K-ras mutation was detected in 3 patients (12%). All three patients did not progressed under chemotherapy. Neither PFS nor OS were affected by K-ras status. The median PFS of all 30 patients was 8.3 months and median OS was 12.7 months. So the authors concluded that there is not correlation between reponses and K-Ras status (15). A Phase II trial assessed the role of Lapatinib in patients with BTC and hepatocarcinoma. Patients with BTC were 17, but no responses were observed and 5 patients had SD. So the authors concluded that lapatinib is not active in BTC (16). These trials showed the possible role of anti-EGFR therapies in patients with BTCs, especially if in combination with chemotherapy or with other biological agents (Table 1). Moreover, many studies have evaluated the incidence of k-ras mutation, with contrasting results in the different reports (mutations of 10-60%), depending also on the site of tumor (17 ,18 , 19 ,20). It still remains unclear whether k-ras mutation is correlated with the response against EGFR inhibitors or not. The biomarker for the response to EGFR inhibitors in BTC, such as the presence of EGFR overexpression, EGFR gene mutation/amplification, or the absence of k-ras mutation, should be investigated in future clinical trials.

3. ANTI-EGFR IN HEPATOCELLULAR CARCINOMA (HCC)

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death. Many treatments have been proposed, but HCC is a chemotherapy-resistant tumor and the median survival for patients with advanced disease is 6-8 months, despite the wide variety of cytotoxic agents tested (21).

On the basis of the molecular mechanisms underlying this disease, new targeted therapies have been tested. Sorafenib has been approved for patients with advanced HCC, after a large Phase III clinical trial demonstrating a significant survival benefit in Child A patients (22). The use of anti-EGFR therapies in HCC is based on the importance of EGFR and its ligands in hepatocarcinogenesis (23) and on EGFR expression by HCC cell lines and tissue (24 , 25) Several studies have demonstrated the overexpression of Epidermal growth factor receptor (EGFR) in the majority of HCC tumor specimens (26 ,27, 28). Recently, the TKI erlotinib has been tested in patients with advanced HCC. A phase II trial evaluated erlotinib in thirty-eight patients with HCC. EGFR/HER1 expression was detected in 88% of the

patients. 32% of patients were progression-free at 6

Table 1. Clinical studies of EGFR inhibitors in BTCs

Author (Reference)	Regimen	Phase	No patients	Line	Results	Comments
Philip et al (10)	Erlotinib	II	42	1°,2°	RR: 7%	EGFR mutation not tested
					PFS: 17%	
Paule B et al (12)	Cetuximab+ GEMOX	II	9	2° (PD after GEMOX)	TTP: 4 months	EGFR, erbB-2, EGFR gene copy number assessed
					OS: 7 months	
Malka D et al (14)	Cetuximab+ GEMOX vs GEMOX	II	101	1°	PFS: 61% vs 44%	Blood/tumor EGFR signalling pathway member assessed
Gruenberger B et al (15)	Cetuximab+ GEMOX	II	30	1°	RR: 63%	No correlation between K-Ras and response
					PFS: 8.3 months	
					OS: 12.7 months	
Ramanathan et al (16)	Lapatinib	II	17	1°/2°	RR:0%	

Abbreviations: EGFR: Epidermal Growth Factor Receptor; NS: Not significant; OS: Overall Survival; PFS: Progression-Free Survival.

months. Three patients had partial radiologic responses of duration of 2, 10, and 11 months, respectively. Disease control was seen in 59% of the patients. Median overall survival time was 13 months (29). Thomas B. *et al* evaluated erlotinib in monotherapy in forty HCC patients. 17 of 40 patients achieved stable disease at 16 weeks of continuous therapy. The PFS at 16 weeks was 43%, and the median overall survival was 43 weeks (10.75 months). No correlation between EGFR expression and outcome was found. The authors concluded that erlotinib prolonged PFS and OS when compared with historical controls (30). Recently a Phase II trial evaluated the combination of cetuximab and erlotinib in patients with advanced HCC. 40 patients were enrolled. The primary end point of PFS after 16 weeks of treatment was 62.5%. Ten patients achieved a partial response for a confirmed overall response rate (intent-to-treat) of 25%. The median PFS event was 39 weeks and the median overall survival was 68 weeks. So the authors concluded that this association has a meaningful antitumor activity, but an additional evaluation in randomized controlled trials is warranted (31).

Regarding cetuximab monotherapy in patients with advanced HCC, a recent Phase II negative study was published. 30 patients received cetuximab monotherapy, but no responses were observed and only 5 patients had stable disease (32).

Another study tested the activity of cetuximab monotherapy in HCC and evaluated serial tumor biopsies for biomarker analyses. 32 patients were enrolled. 27 patients were evaluable for tumor response. Stable disease was achieved in 44.4% (12 patients) for at least 8 weeks of treatment. 55.6% failed to respond to cetuximab (15 patients). The median time to progression for all patients was 8.0 weeks. Preliminary evaluation of surrogate markers showed no correlation with cytogenetic abnormalities based on FISH analyses for chromosome 1 and 8. Furthermore, only 5 of 21 tumor specimens were positive for EGFR expression without gene amplification, evaluated by FISH analyses. Serial tumor specimens are available in 5

responding and in 7 non-responding pts for changes of p27 and p21 expression. p27 and p21 were upregulated simultaneously in 60% (3/5 pts) of responding pts, whereas in patients with treatment failure p27 and p21 expression was detectable in 14% (1/7 pts) only (33). Recently a phase 2 trial of cetuximab in combination with the gemcitabine plus oxaliplatin (GEMOX) regimen was conducted. Forty-five untreated patients with advanced-stage progressive HCC were prospectively enrolled. The confirmed response rate was 20% and disease stabilization was obtained in 40% of patients. The median progression-free and overall survival times were 4.7 months and 9.5 months, respectively. The 1-year survival rate was 40% (34). Comparative randomized trials are warrented to understand the efficacy of this combination in patients with HCC. Table 2 shows clinical trials evaluating targeted agents in HCC.

4. ANTI-EGFR IN PANCREATIC CANCER

Pancreatic cancer is an aggressive disease with a poor prognosis. Gemcitabine has been considered the standard therapy for years, even if only small benefits in patients with advanced disease have been reached (35). The addiction of other chemoterapeutics to gemcitabine has not obtained a significant benefit in survival. So clinical trials have evaluated molecular targeted agents (cetuximab, bevacizumab, farnesyl transferase inhibitors and metalloproteinase inhibitors) in addiction to gemcitabine (Table 3) (36). Regarding anti-EGFR agents clinical trials have evaluated the addiction of erlotinib or cetuximab to standard therapy.

A Phase III clinical trial evaluated the addiction to erlotinib to gemcitabine. 569 patients were randomly assigned. Overall survival based on an intent-to-treat analysis was significantly prolonged on the erlotinib/gemcitabine arm with a hazard ratio of 0.82 (P = .038; median 6.24 months v 5.91 months). One-year survival was also greater with erlotinib plus gemcitabine (23% v 17%; P = .023). Progression-free survival was significantly longer with erlotinib plus gemcitabine with an estimated HR of 0.77 (P = .004) (37). A phase III

Table 2. Clinical studies of EGFR inhibitors in HCC

Author (Reference)	Regimen	Phase	No patients	Results	Comments
Philip et al (29)	erlotinib	II	38	PFS at 6 months 32%	EGFR/HER1 expression detected in 88% of the patients.
				OS: 13 months	
Thomas et al (30)	erlotinib	II	40	PFS at 16 weeks: 43%	No correlation between EGFR expression and outcome
				Median OS: 43 weeks	
Thomas et al (31)	Erlotinib+bevacizumab	II	40	PFS at 16 weeks: 62.5 %	
				Median OS: 68 weeks	
Zhu et al (2007)	Cetuximab	II	30	No response was observed	
Gruenwald et al (32)	Cetuximab	II	32	SD: 44%	No correlation with cytogenetic abnormalities for chromosome 1 and 8. p27 and p21 were upregulated simultaneously in 60% (3/5 pts) of responding patients
				PFS: 8weeks	
Asnacios et al (34)	Cetuximab + GEMOX	II	45	PFS: 4.7 months	
				OS: 9.5 months	
				RR: 20%	

Abbreviations: EGFR: Epidermal Growth Factor Receptor; OS: Overall Survival; PFS: Progression-Free Survival; RR: Response Rate; SD: Stable Disease

Table 3. Main clinical studies of EGFR inhibitors in pancreatic cancer

Author (Reference)	Regimen	Phase	No patients	Results
Moore MJ et al (37)	Erlotinib+gemcitabine VS Gemcitabine	III	569	OS: 6.24 VS 5.91 months
Vervenne et al (38)	Gemcitabine +Erlotinib VS Gemcitabine + erlotinib+ bevacizumab	III	607	PFS: 3.6 VS 4.6 months (p: 0.0002)
				OS: 6 VS 7.1 Months (NS)
Xiong et al (39)	Cetuximab + gemcitabine	II	41 EGFR+	PFS: 3.8 months
				OS: 7.1 months
Philip et al (40)	Cetuximab+gemcitabine VS Gemcitabine	III	735	PFS: 3 VS 3.5 months (NS)
				OS: 6 VS 6.5 Months (NS)
Kindler et al (41)	Gemcitabine + Bevacizumab +Erlotinib VS Gemcitabine + bevacizumab + cetuximab	II	139	PFS: 5.0 VS 5.1 months (NS)
				1 year-OS: 30% VS 35% (NS)

Abbreviations: EGFR: Epidermal Growth Factor Receptor; NS: Not significant; OS: Overall Survival; PFS: Progression-Free Survival.

randomized multicenter study was conducted to evaluate the efficacy and safety of adding bevacizumab to erlotinib and gemcitabine in patients with metastatic pancreatic cancer (AViTA study). 607 patients were enrolled. A significant prolongation of overall survival adding bevacizumab was not observed (P=0.2087), although DFS was significantly improved (from 3.6 to 4.6 months, (P=0.0002). The addiction of Bevacizumab was well tolerated, even if an increase in the incidence of epistaxis, hypertension and proteinuria was reported. This study suggest that an antiangiogenetic therapy can have a role in advanced pancreatic cancer, even if it is imperative identifying patient subgroups that can benefit from this strategy (38).

Regarding cetuximab, a Phase II clinical trial was recently conducted to evaluate the activity of cetuximab and gemcitabine association. The combination showed a promising activity, in fact five patients (12.2%) achieved a partial response, and 26 (63.4%) had stable disease. The median time to disease progression was 3.8 months, and overall survival was 7.1 months (39).

At the 2007 American Society of Clinical Oncology Annual Meeting, Philip *et al.* presented the results of the phase III Southwest Oncology Group (SWOG) S0205 study evaluating the association of gemcitabine and cetuximab versus gemcitabine alone. 766 patients were enrolled. The median survival was 6 months

in the gemcitabine alone arm and 6.5 months in the combination arm (P =0.14). The progression-free survival was 3 months for the gemcitabine alone arm and 3.5 months for the combination arm (P = 0.058). So the study failed to demonstrate a significant advantage of the addition of cetuximab to gemcitabine versus gemcitabine alone (40).

A randomized phase II study evaluated a multi-targeted strategy in Pancreatic cancer patients (n = 139), that received gemcitabine, bevacizumab and erlotinib or gemcitabine, bevacizumab and cetuximab. The authors observed that early hypertension correlated with response. There was no significant difference between the two arms in OS or PFS (41).

5. CONCLUSIONS

Anti-EGFR therapies have shown an impact on patients' prognosis in many cancer types. To date, Phase III randomized clinical studies have not yet demonstrated a specific indication for anti-EGFR therapies in BTCs, pancreatic cancer or HCC. Only erlotinib in association with gemcitabine has shown an impact on PFS in patients with advanced pancreatic cancer, but these results have not had a great clinical impact in daily practice. Most promising drugs tested in Phase II clinical trials are Cetuximab in BTC and HCC and erlotinib in HCC. An important end-point of future studies will be identify which patients are more likely to respond to target therapy, identifying predictive markers of response to anti-EGFR agents. Preliminary studies are evaluating the role of k-Ras and other predictive biomarkers, but prospective studies specifically designed to recognise predictive markers of response are keenly awaited.

6. REFERENCES

1. Yoon JH, GY Gwak, HS Lee, SF Bronk, NW Werneburg, GJ Gores: Enhanced epidermal growth factor receptor activation in human cholangiocarcinoma cells. *J Hepatol*. 41,808-814 (2004)

2. Nakazawa K, Y Dobashi, S Suzuki, H Fujii, Y Takeda, A Ooi: Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. *J Pathol*. 206,356 –365 (2005)

3. Leone F, G Cavalloni, Y Pignochino, I Sarotto, R Ferraris, W Piacibello, T Venesio, L Capussotti, M Risio, M Aglietta: Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. *Clin Cancer Res*. 12 (6), 1680-5 (2006)

4. Ariyama H, B Qin, E Baba, R Tanaka, K Mitsugi, M Harada, S Nakano: Gefitinib, a selective EGFR tyrosine kinase inhibitor, induces apoptosis through activation of Bax in human gallbladder adenocarcinoma cells. *J Cell Biochem*. 97,724-734 (2006)

5. Miyata H, T Sasaki, K Kuwahara, M Serikawa, K Chayama: The effects of ZD1839 (Iressa), a highly selective EGFR tyrosine kinase inhibitor, as a radiosensitiser in bile duct carcinoma cell lines. *Int J Oncol*. 28,915-921 (2006)

6. Zhang Z, AE Sirica: Simultaneous inhibition of ErbB1 and ErbB2 signaling significantly enhances the growth suppression of rat and human cholangiocarcinoma cell lines. *FASEB J*. 21: A71-A72 (2007)

7. Herberger B, W Berger, H Puhalla, K Schmid, S Novak, A Brandstetter, C Pirker, T Gruenberger, M Filipits: Simultaneous blockade of the epidermal growth factor receptor/mammalian target of rapamycin pathway by epidermal growth factor receptor inhibitors and rapamycin results in reduced cell growth and survival in biliary tract cancer cells. *Mol cancer Ther*.8 (6),1547-56 (2008)

8. Wiedmann M, J Feisthammel, T Blüthner, A Tannapfel, T Kamenz, A Kluge, J Mössner, K Caca: Novel targeted approaches to treating biliary tract cancer: the dual epidermal growth factor receptor and ErbB-2 tyrosine kinase inhibitor NVP-AEE788 is more efficient than the epidermal growth factor receptor inhibitors gefitinib and erlotinib. Anticancer Drugs. 17 (7),783-95 (2006)

9. Yoshikawa D, H Ojima, A Kokubu, T Ochiya, S Kasai, S Hirohashi, T Shibata: Vandetanib (ZD6474), an inhibitor of VEGFR and EGFR signalling, as a novel molecular-targeted therapy against cholangiocarcinoma. Br J Cancer. 100 (8), 1257-66 (2009)

10. Philip PA, MR Mahoney, C Allmer, J Thomas, HC Pitot, G Kim, RC Donehower, T Fitch, J Picus, C Erlichman: Phase II study of erlotinib in patients with advanced biliary cancer. J Clin Oncol.. 24, 3069-3074 (2006)

11. Sprinzl MF, CC Schimanski, M Moehler, S Schadmand-Fischer, PR Galle, S Kanzler: Gemcitabine in combination with EGF-receptor antibody (cetuximab) as a treatment of cholangiocarcinoma: A case report. BMC Cancer.6,190 (2006)

12. Paule B, M Bralet, M Herelle, E Rage, M Ducreux, C Guettier and R Adam: Cetuximab plus gemcitabine/oxaliplatin (GEMOX) for patients with unresectable/recurrent intrahepatic cholangiocarcinoma refractory to GEMOX. *J Clin Oncol*. 24 (S18), 14084 (2007)

13. Paule B, MO Herelle, E Rage, M Ducreux, R Adam, C Guettier, MP Bralet: Cetuximab plus gemcitabine-oxaliplatin (GEMOX) in patients with refractory advanced intrahepatic cholangiocarcinomas. *Oncology*. 72, 105-110 (2007)

14. Malka D, T Trarbach, L Fartoux, J Mendiboure, C de la Fouchardière, F Viret, E Assenat, E Boucher, O Rosmorduc, T Greten: A multicenter, randomized phase II trial of gemcitabine and oxaliplatin (GEMOX) alone or in combination with biweekly cetuximab in the first-line treatment of advanced biliary cancer: Interim analysis of the BINGO trial. *J Clin Oncol*. 27,15s, abstr 4520 (2009)

15. Gruenberger B, J Schueller, D Tamandl, K Kaczirek, G Schernthaner, T Gruenberger: K-ras status and response in patients with advanced or metastatic cholangiocarcinoma treated with cetuximab plus gemcitabine-oxaliplatin (GEMOX): a single center phase II study. *J Clin Oncol* . 27,15s, abstr 4586 (2009)

16. Ramanathan RK, CP Belani, DA Singh, M. Tanaka, H J Lenz, Y Yen, HL Kindler, S Iqbal, J Longmate, DR Gandara: Phase II study of lapatinib, a dual inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase 1 and 2 (Her2/Neu) in patients (pts) with advanced biliary tree cancer (BTC) or hepatocellular cancer (HCC) A California Consortium (CCC-P) Trial. *J Clin Oncol.* 24 (suppl 18S):Abstract 4010 (2006)

17. Tannapfel A, M Benicke, A Katalinic, D Uhlmann, F Köckerling, J Hauss, C Wittekind: Frequency of p16 (INK4A) alterations and K-ras mutations in intrahepatic cholangiocarcinoma of the liver. *Gut* 47 (5), 721-727 (2000)

18. Suto T, W Habano, T Sugai, N Uesugi, O Funato, S Kanno, K Saito, S Nakamura: Aberrations of the K-ras, p53, and APC genes in extrahepatic bile duct cancer. *J Surg Oncol* 73 (3),158-163 (2000)

19. Hidaka E, A Yanagisawa, M Seki, K Takano, T Setoguchi, Y Kato: High frequency of K-ras mutations in biliary duct carcinomas of cases with a long common channel in the papilla of Vater. *Cancer Res* 60 (3), 522-524 (2000)

20. Rashid A, T Ueki, YT Gao, PS Houlihan, C Wallace, BS Wang, MC Shen, J Deng, AW Hsing: K-ras mutation, p53 overexpression, and microsatellite instability in biliary tract cancers: a population-based study in China. *Clin Cancer Res* 8 (10), 3156-3163 (2002)

21. Simonetti RG, A Liberati, C Angiolini, L Pagliaro: Treatment of hepatocellular carcinoma: A systematic review of randomized controlled trials. *Ann Oncol* 8:117-136 (1997)

22. Llovet JM, S Ricci, V Mazzaferro, P Hilgard, E Gane, JF Blanc, AC de Oliveira, A Santoro, JL Raoul, A Forner, M Schwartz, C Porta, S Zeuzem, L Bolondi, TF Greten, PR Galle, JF Seitz, I Borbath, D Häussinger, T Giannaris, M Shan, M Moscovici, D Voliotis, J Bruix; SHARP Investigators Study Group: Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 359:378-390 (2008)

23. Schiffer E, C Housset, W Cacheux, D Wendum, C Desbois-Mouthon, C Rey, F Clergue, R Poupon, V Barbu,O Rosmorduc: Gefitinib, an EGFR inhibitor, prevents hepatocellular carcinoma development in the rat liver with cirrhosis. *Hepatology.* 41:307–314 (2005)

24.   Ito Y, T Takeda, M Sakon, M Tsujimoto, S Higashiyama, K Noda, E Miyoshi, M Monden, N Matsuura: Expression and clinical significance of erb-B

receptor family in hepatocellular carcinoma. *Br J Cancer.* 84:1377–1383 (2001)

25. Huether A, M Hopfner, V Baradari, D Schuppan, H Scherubl: EGFR blockade by cetuximab alone or as combination therapy for growth control of hepatocellular cancer. *Biochem Pharmacol*. 70:1568–1578 (2005)

26. Buckley AF, LJ Burgart, S Kakar: Epidermal growth factor receptor expression and gene copy number in fibrolamellar hepatocellular carcinoma. *Hum Pathol* 37:410-414 (2006)

27. Hamazaki K, Y Yunoki, H Tagashira, T Mimura, M Mori, K Orita: Epidermal growth factor receptor in human hepatocellular carcinoma. *Cancer Detect Prev* 21:355-360 (1997)

28. Nakopoulou L, K Stefanaki, D Filaktopoulos, I Giannopoulou: C-erb-B-2 oncoprotein and epidermal growth factor receptor in human hepatocellular carcinoma: An immunohistochemical study. *Histol Histopathol* 9:677-682 (1994)

29. Philip PA, MR Mahoney, C Allmer, J Thomas, HC Pitot, G Kim, RC Donehower, T Fitch, J Picus, C Erlichman: Phase II study of erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *J Clin Oncol* 23:6657-6663 (2005)

30. Thomas MB, R Chadha, K Glover, X Wang, J Morris, T Brown, A Rashid, J Dancey, JL Abbruzzese: Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. *Cancer* 110:1059-1067 (2007)

31. Thomas MB, JS Morris, R Chadha, M Iwasaki, H Kaur, E Lin, A Kaseb, K Glover, M Davila, J Abbruzzese: Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. *J Clin Oncol*. 20;27 (6):843-50 (2009)

32. Zhu AX, K Stuart, LS Blaszkowsky, A Muzikansky, DP Reitberg, JW Clark, PC Enzinger, P Bhargava, JA Meyerhardt, K Horgan, CS Fuchs, DP Ryan: Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. *Cancer*. 1;110 (3):581-9 (2007)

33. Gruenwald V, L Wilkens, T Gebel, T Wirth, T Greten, S Kubicka, MP Manns, A Ganser, NP MalekL: A phase II openlabel study of cetuximab in unresectable hepatocellular carcinoma: final results (abstract) *Proc Am Soc Clin Oncol*. 25:4598a (2007)

34. Asnacios A, L Fartoux, O Romano, C Tesmoingt, S S Louafi, T Mansoubakht, P Artru, T Poynard, O Rosmorduc, M Hebbar, J Taieb: Gemcitabine plus oxaliplatin (GEMOX) combined with cetuximab in patients with progressive advanced stage hepatocellular carcinoma: results of a multicenter phase 2 study. *Cancer*. 15;112 (12):2733-9 (2008)

35.Burris HA 3rd, MJ Moore, J Andersen, MR Green, ML Rothenberg, MR Modiano, MC Cripps, RK Portenoy, AM

Storniolo, P Tarassoff, R Nelson, FA Dorr, CD Stephens, DD Von Hoff: Improvements in survival and clinical benefit with gemcitabine as firstline therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 15:2403-13 (1997)

36. Strimpakos A, MW Saif, KN Syrigos. Pancreatic cancer: from molecular pathogenesis to targeted therapy. *Cancer Metastasis Rev* 27(3):495-522 (2008)

37. Moore MJ, D Goldstein, J Hamm, A Figer, JR Hecht, S Gallinger, HJ Au, P Murawa, D Walde, RA Wolff, D Campos, R Lim, K Ding, G Clark, T Voskoglou-Nomikos, M Ptasynski, W Parulekar: Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007, 25:1960-1966.

38. Vervenne W, J Bennouna, Y Humblet, S Gill, MJ Moore, J Van Laethem, A Shang, J Cosaert, C Verslype, E Van Cutsem: A randomized, double-blind, placebo (P) controlled, multicenter phase III trial to evaluate the efficacy and safety of adding bevacizumab (B) to erlotinib (E) and gemcitabine (G) in patients (pts) with metastatic pancreatic cancer. *J Clin Oncol* (Meeting Abstracts) 2008, 26:4507.

39. Xiong HQ, A Rosenberg, A LoBuglio, W Schmidt, RA Wolff, J Deutsch, M Needle, JL Abbruzzese: Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II trial. *J Clin Oncol*. 22 (13):2610-2616 (2004)

40. Philip PA, J Benedetti, C Fenoglio-Preiser, M Zalupski, H Lenz, E O'Reilly, R Wong, J Atkins, J Abruzzese, C Blanke: Phase III study of gemcitabine (G) plus cetuximab (C) versus gemcitabine in patients (pts) with locally advanced or metastatic pancreatic adenocarcinoma (PC): SWOG S0205 study. *ASCO Annual Meeting Proceedings* (Post- Meeting Edition) Part I. 25: (18S June 20 suppl):LBA4509 (2007)

41. Kindler HL, T Gangadhar, T Karrison, HS Hochster, MJ Moore, K Micetich, W Sun, DV Catenacci, WM Stadler, EE Vokes: Final analysis of a randomized phase II study of bevacizumab (B) and gemcitabine (G) plus cetuximab (C) or erlotinib (E) in patients (pts) with advanced pancreatic cancer (PC) *J Clin Oncol* (Meeting Abstracts) 26:4502 (2008.)

Send correspondence to: Daniele Santini, Medical Oncology, University Campus Bio-Medico, Via Alvaro del Portillo, 200, 00128 Rome, Italy, Tel:0039-06-225411206, Fax: 0039-06-225411638, E-mail: d.santini@unicampus.it