CASE AND REPORTS

Fish protein extract enhances clinical response to salvage chemotherapy in colon cancer patients

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Abstract

Salvage treatment for advanced colon cancer, despite a high rate in objective clinical response, provides no significant benefit in terms of increased survival when compared to supportive care. Zebrafish embryo extracts have been shown to re-activate apoptotic mechanisms in resistant cancer cells and to counteract chemotherapy-induced side effects, both in in vitro and in vivo studies. The present pilot, observational trial compares both safety and overall survival in two randomized groups of advanced colon cancer patients treated with Regorafenib as salvage therapy versus Regorafenib plus Synchro Levels®, a food supplement containing a peptide mix. At 12 months, a statistically significant increase in survival was observed in the latter group (75% versus 33.3%). Similarly, in Synchro Levels® treated patients Performance Status is largely preserved during the treatment, whereas it declines rapidly in the Regorafenib group. Overall, this data suggests that Synchro Levels® may improve the clinical response of salvage therapy, by both modulating drug responsiveness and counteracting drug-related side effects. Further larger, randomized trials are warranted to confirm such promising preliminary results.

Keywords: side effects of cancer treatment; Synchro Levels®; Regorafenib; Drug Resistance; Colon Cancer; Tumor reprogramming

1. Introduction

Colorectal cancer is the third most commonly diagnosed cancer with around one million new cases every year and a major cause of mortality throughout the world (Malvezzi 2011). Patients with advanced metastatic disease are usually eligible for multiple lines of treatment. Three major chemotherapeutic agents (5-fluorouracil (5-FU), irinotecan and oxaliplatin), one anti-vascular endothelial growth factor (anti-VEGF, bevacizumab) and two epidermal growth factor receptor inhibitors (cetuximab and panitumumab) have shown clinical activity for the treatment of metastatic colorectal cancer. Most patients receive the three chemotherapeutic agents during their first two lines of treatment (Van Cutsem 2010) and the simultaneous administration of the three drugs has been demonstrated to evoke a higher response when employed as first line treatment (Falcone 2007). Phase III trials reported the efficacy of bevacizumab in the first or second line, but

there are no data supporting its prescription beyond the second line (Hurwitz 2004). Anti-epidermal growth factor receptor drugs (cetuximab, panitumumab) have been demonstrated to be clinically active in third-line treatment, even if they are frequently used in first- and second-line treatment too (Peeters Price 2010). However, despite some exciting results recorded mostly as short-term tumor response rate, prognosis remains poor for most of these patients. In the case of disease progression after two lines of treatment, no significant differences were recorded in the overall survival rates among patients treated with panitumumab or supportive care alone (Van Cutsem 2007). Cetuximab has been credited for inducing higher overall survival (OS) in chemotherapy-refractory colon cancer patients (Jonker 2007). Although such an assertion builds upon reliable statistical analysis, a median OS of 6.1 months in the Cetuximab group versus 4.6 months observed in the control group cannot be seriously considered an "advancement". Indeed, the use of these monoclonal antibodies is severely limited by the presence of intrinsic drug resistance mechanisms or by the ability of cancer cells to acquire de novo resistance. To overcome drug resistance in heavily treated cancer patients, new drugs have been approved for metastatic colon cancer and are still under scrutiny. In 2012, the US FDA licensed Regorafenib, a multi-kinase inhibitor, and this drug is now standard in patients who have failed all other standard therapies. Nevertheless, even in this case, clinical results showed little benefit, if any. In Regorafenib-treated patients, an OS of 6.4 months was observed, longer than the 5.0 months obtained in the placebo group (Grothey 2013). Again, we are faced with very modest improvements, considering that the median progression-free survival was 1.9 months for Regorafenib and 1.7 months for a placebo. Furthermore, Regorafenib was associated with significant adverse effects - mainly fatigue, hypertension, hand–foot syndrome, diarrhoea and cutaneous rash – occurring in up to 90% of patients. In fact, nearly all (>95%) treated-patients with multi-kinase inhibitors experience at least one side effect of any grade of severity (Sodergren 2014).
Overall, clinical data suggest that in those refractory colon cancer patients having received two lines of chemotherapy, further available treatments (with monoclonal antibodies or kinase inhibitors with or without antineoplastic drugs) can increase OS obtained with the best supportive care by just a few months. Therefore, current therapeutic options are mainly focused on modelling the 'best therapeutic sequences', depending on previously used therapies, on the patient and the tumor biology (Foubert 2014). Yet, even these attempts are likely to fail if drug resistance is not efficiently counteracted.

2. Experimental and molecular studies with zebrafish extracts

Compelling evidence has demonstrated that differentiating factors as well as soluble morphogens extracted from embryonic, pluripotent cells or from stem cells may efficiently inhibit cancer growth, promote apoptosis or induce phenotypic tumor reversion, documented by morphological, biochemical, behavioral as well as metabolomic features (reviewed in Bizzarri 2011). Zebrafish extracts have been proven to modulate several critical pathways (including the p53 and the pRB pathways), enhancing apoptosis and inhibition of cell growth, ultimately leading to cancer cell differentiation into a less malignant or even 'normal', 'reversed' phenotype (Biava 1988; Cucina 2006; Biava 2002; Biava 2001; D'Anselmi 2011). As suggested since the 70s by Pierce "It is now clear that the embryonic fields can regulate their closely related malignant cell types, and thus it is our hypothesis that there must be an embryonic field capable of regulating every carcinoma"(Pierce 1971). In fact, it has recently been shown that implantation of melanoma cells into Zebrafish embryos does not result in tumor development; while in the adult animal a tumor is formed (Topczewska 2006).

Moreover, injection of melanoma cells in Zebrafish extra-embryonic membranes originated Zebrafish neuronal cells. This demonstrates that cancer cells can differentiate in normal tissues when implanted in embryos (Kulesa 2006). Noteworthy, zebrafish extracts were able to reverse the chemotherapy-induced drug-resistance in colon cancer cells by down-regulating several anti-apoptotic factors (Bizzarri 2011). In fact, zebrafish extracts in colon cancer cells induced an almost complete suppression of Bcl-xL release and a dramatic increase in the Bax/Bcl-xL ratio, thus suggesting this treatment could efficiently improve chemotherapy efficacy by reducing anti-apoptotic proteins involved in drug resistance processes (D'Anselmi 2011).

2.1 Zebrafish embryo extracts display anticancer effects

Up to now, Zebrafish extracts have been investigated in clinical settings in only a few pilot studies. A preliminary randomized clinical trial with zebrafish extracts on 179 patients with intermediate-advanced hepatocellular carcinoma (HCC) unresponsive to conventional treatment (transplantation, resection, ablation therapy or chemoembolization) provided valuable and interesting results (Livraghi 2005). Zebrafish extracts in microgram concentrations were delivered sublingually at a dose of 30 drops three times-a-day for each patient during a period ranging from six to forty months. Regression occurred in 19.8% of the patients (2.4% of complete regression, and 17.4% of partial regression) and disease stabilization in 16% of patients. The overall survival rate was over 60% survival rate after forty months in responding patients, while survival was less than 10% in non-responding subjects. Furthermore, performance status (PF) improved in 82.6% of treated patients, including those in whom the tumor had progressed. Treatment with zebrafish extract was devoid of any significant side effects. A more recent clinical trial conducted on 50 patients with advanced stage HCC provides a substantial confirmation of those results (Livraghi 2011). Indeed, in 13.1% of advanced HCC patients a long-lasting complete response was observed, together with a significant down-regulation of α-fetoprotein (AFP). Furthermore, a pilot study of six heavily pre-treated advanced HCC patients in which palliative treatment with zebrafish extract (0.02 g three times a day for 9 months) was administered, provided further evidence in support of these previous data (Franchi 2005). Performance status improved in all patients but one, while disease remained stable (only one patient showed a slight progression). Interestingly, in patients with stable disease a remarkable reduction in AFP levels was recorded following the first three months of treatment. However, it is quite hard to fathom why those patients achieved such a remarkable response. No single active component has been hitherto identified within the crude protein extract from the Zebrafish embryo, or from other conditioned embryo milieu, that could explain the observed pharmacological effect. An extensive search for those anti-cancer factors has been performed (Biava 2015). Yet, no conclusive data have been provided and the identification of embryo-related anticancer molecular effectors is still a matter of intensive research. Additionally, zebrafish embryo extracts may also exert their effects by modifying some biophysical cues, as well as through soluble morphogens, chiefly involving the cell-microenvironment cross talk [Abbott 2008; Bischof 2013; Telerman 2009). Both these mechanisms could act by reshaping the morphogenetic field in which cancer cells are embedded, namely by counteracting the chemotherapy-induced drug-resistance (Huang 2007). Indeed, it is widely agreed that further improvement in outcomes for advanced colon cancer will depend on better patient selection and on identifying and targeting mechanisms of drug resistance (Fakih 2015). Given that Zebrafish extracts have shown to re-activate apoptotic pathways in resistant colon cancer cell lines (D'Anselmi 2011), we undertook a pilot observational study to investigate if such effect could counteract drug-resistance induced by Regorafenib-based, 'salvage therapy' in advanced colon cancer patients.

3. Material and Methods

3.1 Colon cancer patients

Patients affected by advanced metastatic colon cancer, heavily pretreated with two different chemotherapy protocols (first and second line treatment) were included in the trial to receive Regorafenib-based salvage treatment. Patients were neither eligible for further surgical intervention (because of the size and number of their lesions), nor were they eligible for radiation therapy. Additionally, they had previously experienced relevant side effects in a not negligible proportion of cases (20 out of 24 patients). Patients were randomly distributed by the closed-enveloped method into two groups: a) Regorafenib-only (RE) treated patients (n. 12); and, b) Regorafenib + Syncro Levels® treated patients (n. 12). Syncro Levels® is a food supplement containing peptide mix. Exclusion criteria were: age over 70 years, uncontrolled liver disease, renal failure, terminal stage reflecting a life expectancy shorter than three months or an ECOG performance status (0-10) greater than 5. Both groups received Regorafenib 160 mg/day p.os for three weeks with a rest of one week. In the experimental arm, an oral sublingual dose of 1 ml Syncro levels® was added three times a day. The treatment was discontinued in the presence of untreatable side effects or after tumor progression. Tumor evolution was scrutinized every six months by means of TC scan. Clinical examination as well as laboratory biochemical analysis were executed

every three months. The primary outcome measure was tumor response, determined according to EORT/NCI criteria (RECIST) (Nishino 2010). In addition, overall survival between the two groups was noted every three months. The secondary outcome was the performance status and patient tolerance, monitored every three months. A detailed recording of different side effects (headache, muscle fatigue, edema and facial edema, nausea and vomiting, respiratory distress, diarrhea, abdominal pain, constipation, anorexia, swallowing, reflux, weight loss, itchy skin, skin rash, mucositis, stomatitis, alopecia, joint pain, infection, miscellaneous) was obtained for each patient. Patients were provided with an information sheet explaining the nature of Syncro levels® and possible benefit derived from the product. They were recruited for the study after signing the informed consent form.

3.2 Statistical analysis

Differences in survival rate were analyzed by means of Chi-Square test. Changes in Performance Status values were investigated through a non-parametric approach (i.e., Wilcoxon test), analysis more suited for rank variables, will be conducted. Principal Component Analysis allowed us to generate a global quality of life index (factor1, see Supplementary Material) that was in turn used to check for quality of life comparison of the two experimental groups.

4. Results

4.1 Control group

Patient features and distribution among the two groups, as well as the clinical response data are reported in Table 2, while Tab. 1 reports the incidence and distribution of side effects (including weight loss) between the two patient groups, after 3 and 9 months of treatment. Within the control group treated with Regorafenib only, 2 out of 12 patients (16.6%) showed a complete objective response during the first six months, while a partial response was observed in eight patients (66.6%).

Side effects	RE group M3	RE group M9	ZFE group M3	ZF group M9
Constipation	1	1	1	1
Diarrhoea	1	3	-	-
Weight loss (>10%)	1	4	-	1
Headache	-	1	1	1
Muscle fatigue	2	3	-	1
Edema	-	-	1	-
Nausea/ vomiting	4	4	1	1
Respiratory distress	1	1	1	-
Itchy skin	1	-	-	-
Skin rash	-	1	-	-
Mucositis/ stomatitis	1	3	-	-
Alopecia	3	3	2	2
Swallowing/ reflux	1	1	-	-
Joint pain	-	1	-	1
Infection	-	1	-	-

Table 1. Incidence and distribution of side effects between the two groups of patients, after 3 and 9 months of treatment. Analysis was not extended to 12 months given that at this time most patients in the RE group were almost all dead.

Raw data of median survival showed that patients in Syncro levels® group survived 11.5 in months versus 9.3 in the RE arm. At 12 months, only three patients in the RE group were still responding, while one patient was in progression and still alive. The remaining nine patients died after nine to twelve months. Eleven patients discontinued the treatment between the eighth and ninth month because of progression or for relevance of occurring side effects.

	n. pts	sex	age	PF M0	PF M3	PF M6	PF M9	PF M12	T6	T12	Surv. M3	Surv. M6	Surv. M9	Surv M12
Synchro Levels® group	1	M	66	3	3	4	3	3	PR	CR	Y	Y	Y	Y
	2	M	67	4	3	3	3	3	CR	CR	Y	Y	Y	Y
	3	F	58	4	3	3	2	2	PR	PR	Y	Y	Y	Y
	4	F	58	5	3	3	3	3	NC	PR	Y	Y	Y	Y
	5	F	51	4	3	2	2	2	NC	PR	Y	Y	Y	Y
	6	F	55	4	4	3	3	3	NC	PR	Y	Y	Y	Y
	7	F	65	4	3	3	3	3	PR	PR	Y	Y	Y	Y
	8	M	55	5	3	3	3	4	PR	P	Y	Y	Y	Y
	9	M	59	3	4	6	7	dead	NC	dead	Y	Y	dead	dead
	10	M	61	3	3	4	4	4	NC	NC	Y	Y	Y	Y
	11	F	58	4	3	5	7	dead	P	dead	Y	Y	Y	dead
	12	M	59	4	3	4	5	dead	NC	Dead	Y	Y	Y	dead
RE	1	M	66	3	4	4	5	dead	PR	dead	Y	Y	y	dead
	2	M	66	4	5	6	dead	dead	PR	dead	Y	Y	dead	dead
	3	M	56	4	5	4	7	7	CR	P	Y	Y	Y	Y
	4	M	67	5	4	4	7	7	CR	PR	Y	Y	Y	Y
	5	F	59	5	6	5	5	5	PR	dead	Y	Y	Y	dead
	6	F	63	5	4	4	5	5	PR	PR	Y	Y	Y	Y
	7	M	62	3	3	4	5	4	NC	dead	Y	Y	Y	dead
	8	F	63	4	4	5	6	dead	PR	dead	Y	Y	Y	dead
	9	M	67	4	4	6	7	dead	PR	dead	Y	Y	Y	dead
	10	M	59	3	4	5	6	7	PR	dead	Y	Y	Y	dead
	11	M	66	3	4	5	6	dead	P	dead	Y	Y	dead	dead
	12	F	55	4	3	3	3	5	PR	PR	Y	Y	Y	Y

Table 2. Incidence and distribution of side effects between the two groups of patients, after 3 and 9 months of treatment. Analysis was not extended to 12 months given that at this time most patients in the RE group were almost all dead.

4.2 Experimental arm

During the first six months, an objective response (1 CR and 4 PR) was documented in 5 out of 12 patients (66.5%). Six patients were in a stable condition (NC) whereas one was in progression. At 12 months, two complete regressions were recorded (16.6%) while five patients still demonstrated an objective partial regression. Overall, responding patients accounted for 58.3%. Three patients died between the ninth and twelve months, while the remaining patient was still alive but in progression. Treatment was discontinued only in this case. Overall, at 12 months, the association of Regorafenib with Synchro levels® induced a long-lasting regression in seven patients (58.3%) with an overall survival of 75%. In the control group, after 12 months, no complete regression was observed, while only four patients were still alive (33.3%).

These results are reported in the 2x2 contingency table (Table 3) below.

Notwithstanding the low number of patients, this difference is statistically significant in Chi-Square test (p<0.04). Usually, the Kaplan-Meier + log rank test rep-

resents the 'elective' test for the comparison of survival rates but looking at Table 1 reporting the actual data set it is evident all three death events occurred at 12 months. Thus, the actual information the Kaplan-Meyer model should be based on, practically corresponds to a 'two-point' regression on the 'dead/alive at 12 months' binary outcome. The same clearly holds for the log-rank test that (due to the high number of ties) should collapse as well to the alive/dead at 12 months information that is actually the information we processed here using the Chi-Square test. Indeed, the Chi-Square test is the obvious way to compare the two groups. In any case we performed Kaplan-Meier analysis obtaining $z = 2.27$ and a statistically significant log-rank test at $p = 0.023$.

Treatment	Dead (n. pts)	Alive (n. pts)
Regorafenib	8	4
Fish Protein Extract	3	9

Table 3. Patients Survival at 12 months

4.3 Performance status

Incidence and distribution of side effects were reported in Tab.2, while Tab.4 describe the average values of mean biochemical parameters measured in both patient groups at 3 and 9 months of treatment. Overall, several hematological parameters (namely HGB, proteins, RBC and WBC) show to be improved on average in the Syncro levels® group after 9 months of treatment, even if no clear conclusion can be obtained from a statistical point of view. Analysis was not extended to 12 months given that at this time most patients in the RE group were almost all dead. A) Control group. Regorafenib therapy was discontinued after eight months in all patients, due to cumulative toxicities. On average, each patient received 6.8 courses of salvage therapy. In the control group (RE), treated with only Regorafenib, the performance status (PF) worsened even during the first three months, increasing from a median value of 3.1 to 5.2. This value did not change significantly at 6 and 12 months (5.2 and 5.5 respectively) in the surviving patients. Patients in progression were not considered as their PF increased abruptly, ultimately leading to death. B) Experimental arm. In the salvage therapy + Syncro levels® group, patients received on average 9.1 courses of salvage therapy and Zebrafish extracts. An opposite trend was ascertained in the experimental

arm where PF value decreased from a median value of 3.2 to 2.9 after three months. Indeed, side effect incidence was significantly low in this group (data not shown). No significant changes in PF value were recorded at 6 and 12 months (3.1 and 3.3 respectively) given that PF remain stable. Wilcoxon test, as applied to PF3-PF12 demonstrated a statistically significant difference between the two groups in PF3 (p<0.001), PF6 (p < 0.02), PF9 (p<0.01) and PF12 (p<0.005), while the two groups have a non-statistically significant difference as for starting condition (PF0). When submitted to principal Component Analysis (PCA), PF evolution in time gave rise to two independent factors: Factor 1 (PF value during the treatment period, explaining 58% of total variance) and Factor2 (the starting PF value, explaining around 30% of variance). The Factor1-Factor2 space is a bona fide (88% of total variance explained) complete description of the quality of life space recorded during the trial. The two groups are significantly different (t-test below) as for Factor1 (treatment period) while identical for Factor2 (starting condition).

This is the 'ideal' situation: the treatment with Syncro levels® and Regorafenib (Syncro levels ® group) drastically ameliorates the quality of life when compared to Regorafenib only (RE), as we had already seen in the previous results (Wilcoxon test) on a month-by-month basis. Principal components are independent of each other by construction on the entire data set (RE + Syncro levels® group) this implies that the four quadrants of the bi-dimensional space spanned by Factor1 and Factor2 generated by the means of the two components are expected to host the same number of patients (Figure.1).
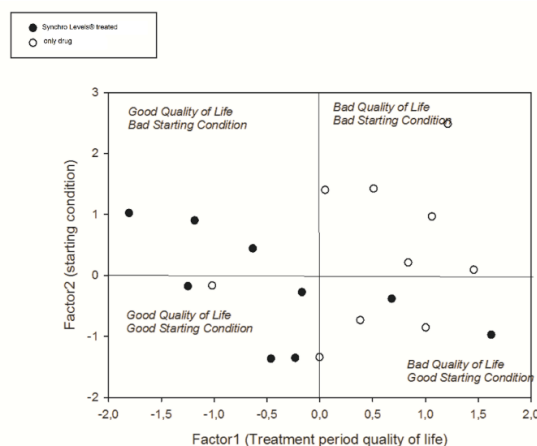
It is noteworthy that the first quadrant (Left, Top, corresponding to patients having a poor initial condition but reaching a good quality of life during treatment) includes only Syncro levels® treated patients, while quadrant number 2 (Top, Right, bad initial conditions remaining bad during treatment) comprised RE-treated patients only.

5. Discussion

The carcinogenic paradigm on which current treatment strategy relies is challenged by shortcomings (Weinberg 2014) and alternative theories (Bizzarri 2008) and, above all, by the substantial failure of current drug-based treatments (Wise 2016).

Indeed, despite exciting advances in targeted therapies, high drug costs, marginal therapeutic benefits, and notable toxicities are concerning aspects of today's cancer treatments (Wheatly 2014). Furthermore, driven by the inherent heterogeneity of cancers, resistance too often leaves single-target strategies with diminished efficacy, and overall survival is limited or not improved at all (Wise 2016; Heppt 2015).

This is especially true in heavily pretreated advanced colon cancer patients eligible for salvage therapy (Van Cutsem 2007; Jonker 2007; Grothey 2013). Herein we performed a pilot, randomized study in which advanced colon cancer patients were randomized into two groups receiving Regorafenib-based salvage therapy only or a protocol in which Syncro levels® containing peptide mix was associated with Regorafenib.

Figure 1. The figure reports the projection of the patients in the first two components (Factor1, Factor2) space. The x-axis corresponds to Factor1 explaining (see Supplementary Material) the 58% of total variance of the PF0-PF9 space. Factor1 is strongly related with PF3-PF9 variables and independent from PF0, thus it represents a global cumulative score of the quality of life during treatment period. Y-axis corresponds to Factor2 scores (29.1% of explained variance). Factor2 correlates near unity (r = 0.91, see Supplementary Materials) with PF0, thus it corresponds to the quality of life at the starting condition. Factors have by construction zero mean and unit standard deviation, thus the entire patient space can be subdivided into four quadrants centered on the zero mean. The four quadrants have the meaning reported in the figure directly descending from the factors loading pattern. It is worth noting the patients reaching a good quality of life starting from bad initial conditions (top left quadrant) are only relative to the Synchro Levels® group.

Embryo or oocyte-derived factors have been demonstrated to orchestrate a complex anticancer action, ultimately leading to cancer 'rewiring', with 'normalization' of the malignant phenotype (Cucina 2006; Biava 2002; Biava 2001; D'Anselmi 2011; D'Anselmi 2013). The strategy based on 'tumor reversion' has gained momentum during the last decade, given that some molecular effectors supporting this phenotypic transition have come to light (Telerman 2010).

Furthermore, embryo extract may prevent the emergence of drug-resistance [16], thus enhancing the anticancer effects of conventional treatments. This hypothesis received a promising confirmation from results of the present trial. Indeed, patients treated with Regorafenib and Syncro levels® experienced a significantly higher number of long-lasting remissions and they were predominantly alive at 12 months. Survival rate accounts for 75% in the Syncro levels® group, while only 33.3% of Regorafenib-only treated patients still survived after one year. Furthermore, Syncro levels®-treated patients experienced fewer side effects than Regorafenib-only treated patients, and were able to preserve their performance status or even to improve it.

Notwithstanding the small number of patients, this difference is highly significant. We hypothesize that such an effect can be mostly ascribed to two major factors: a) zebrafish extract may have enhanced the rescue of pro-apoptotic mechanisms – as previously shown in in vitro studies (Bizzarri 2011; D'Anselmi 2011) – thus improving the tumor response to Regorafenib. b) Zebrafish in counteracting drug-related side effects, as previously observed in other malignancies (Bizzarri 2002), enables patients receiving a significantly higher number of treatment courses (9.1 versus 6.8 in the RE group), thus enhancing the anticancer effect of the conventional drug.

Limitations of the present study obviously relate to the limited number of patients. Therefore, a wide-randomized trial is warranted to confirm those preliminary, intriguing results.

6. Conclusion

The treatment options for both first-line and second-line metastatic colorectal cancer in the modern era include combination chemotherapy and/or biologics, among which is Regorafenib, an oral tyrosine kinase inhibitor. Yet, even these new drugs lead to minimal benefits with increased toxicities.

However, adding Syncro levels® to salvage Regorafenib-based therapy may significantly reduce the burden of side effects, while amplifying the clinical response.

Patients treated with Regorafenib and Syncro levels® experienced long-lasting remission with increased survival.

Such results may disclose new opportunities in salvage treatment strategies for advanced colon cancer. Data herein reported highlight the relevance of innovative hybrid approaches which "can exploit advantages of both non-conventional and modern medicine Moreover, the integrative treatment approach suggest that conventional treatments are inadequate in addressing the complexity of cancer cure, when they are only focused in obtaining 'objective' (i.e., tumor) response rate, while disregarding the well-being of patients. Indeed, our results confirmed the "discrepancy between "disease care" and "health care" which dramatically impacts ethical and economical aspects of medical services.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abbott DE, Bailey CM, Postovit LM, Seftor EA, Margaryan N, Seftor RE, Hendrix MJ, 2008, The epigenetic influence of tumor and embryonic microenvironments: How different are they? *Cancer Microenviron*, vol. 1, pag. 13-21.

Biava PM, Bonsignorio D, Hoxha M, Facco R, Ielapi T, Frati L, Bizzarri M, 2002, Post-translational modifications of the retinoblastoma protein (pRB) induced by in vitro administration of Zebrafish embryonic extracts on human kidney adenocarcinoma cell line. *J. Tumor Marker Oncol*, vol. 17(3), pag. 59-64.

Biava PM, Canaider S, Facchin F, Bianconi E, Ljung-berg L, Rotilio D, Burigana F, Ventura C, 2015, Stem Cell Differentiation Stage Factors from Zebrafish Embryo: A Novel Strategy to Modulate the Fate of Normal and Pathological Human (Stem) Cells, *Curr Pharm Biotechnol*, vol. 16(9), pag. 782-792.

Biava PM, Fiorito A, Negro C, Mariani M, 1988, Effects of treatment with embryonic and uterine tissue homogenates on Lewis lung carcinoma development, *Cancer Lett*, vol. 41, pag. 265-270.

Biava, PM, Bonsignorio D, Hoxha M, 2001, Cell proliferation curves of different human tumor lines after in vitro treatment with Zebrafish embryonic ex-tracts. *J. Tumor Marker Oncol*, vol. 16, pag. 195-201.

Bischof AG, Yüksel D, Mammoto T, Mammoto A, Krause S, Ingber DE, 2013, Breast cancer normalization induced by embryonic mesenchyme is mediated by extracellular matrix biglycan. *Integr Biol (Camb)*, vol. 5(8), pag. 1045-1056.

Bizzarri M, Cucina A, Biava PM, Proietti S, D'Anselmi F, Dinicola S, Pasqualato A, Lisi E, 2011, Embryonic morphogenetic field induces phenotypic reversion in cancer cells. *Curr Pharm Biotechnol*, vol. 12, pag. 243-253.

Bizzarri M, Cucina A, Conti F, D'Anselmi F, 2008, Beyond the oncogene paradigm: understanding complexity in carcinogenesis. *Acta Biotheor*, vol. 56, pag. 173-196.

Bizzarri M, Facco R, Ielapi T, Frati L, 2002, The embryonic and maternal regulatory factor as a palliative therapy for advanced solid tumours, *J. Tumour Marker Oncol*, vol. 17(3), pag. 31-36.

Cucina A, Biava PM, D'Anselmi F, Coluccia P, Conti F, di Clemente R, Miccheli A, Frati L, Gulino A, Bizzarri M, 2006, Zebrafish embryo proteins induce apoptosis in human colon cancer cells (Caco2), *Apoptosis*, vol. 11, pag. 1617-1628.

D'Anselmi F, Cucina A, Biava PM, Proietti S, Coluccia P, Frati L, Bizzarri M, 2011, Zebrafish stem cell differentiation stage factors suppress Bcl-xL release and enhance 5-Fu-mediated apoptosis in colon cancer cells, *Curr Pharm Biotechnol*, vol. 12(2), pag. 261-267.

D'Anselmi F, Masiello MG, Cucina A, Proietti S, Dinicola S, Pasqualato A, Ricci G, Dobrowolny G, Catizone A, Palombo A, Bizzarri M, 2013, Microenvironment promotes tumor cell reprogramming in human breast cancer cell lines, *PLoS One*, vol. 8(12), pag. e83770.

Fakih MG, 2015, Metastatic Colorectal Cancer: Cur-rent State and Future Directions, *J Clin Oncol*, vol. 33(16), pag. 1809-1824.

Falcone A, Ricci S, Brunetti I, Pfanner E, Allegrini G, Barbara C, Crinò L, Benedetti G, et al., 2007, Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. *J Clin Oncol*, vol. 25, pag. 1670–1676.

Foubert F, Matysiak-Budnik T, Touchefeu Y, 2014, Options for metastatic colorectal cancer beyond the second line of Treatment, *Dig Liver Dis*, vol. 46(2), pag. 105-112.

Franchi F, Ielapi T, Bizzarri M, Seminara P, 2005, Embryo extracts opotherapy reduces alpha-fetoprotein levels in hepatocellular carcinoma patients. *J Gastroenterol Hepatol*, vol. 20, pag. 1467-1468.

Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, et al., 2013, CORRECT Study Group. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an inter-national, multicentre, randomised, placebo-controlled, phase 3 trial, *Lancet*, vol. 381, pag. 303–312.

Heppt MV, Tietze JK, Graf SA, Berking C, 2015, Combination therapy of melanoma using kinase inhibitors, *Curr Opin Oncol*, vol. 27, pag. 134-140.

Huang S, Ingber D, 2006-2007, A non-genetic basis for cancer progression and metastasis: self-organizing attractors in cell regulatory networks. *Breast Dis*, vol. 26, pag. 27-54.

Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, et al., 2004, Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*, vol. 350, pag. 2335–2342.

Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahn M, et al., 2007, Cetuximab for the treatment of colorectal cancer. *N Engl J Med*, 357, 2040–2048.

Kulesa PM, Kasermeier-Kulesa JC, Teddy JM, Margaryan NV, Seftor EA, Seftor RE, Hendrix MJ, 2006, Reprogramming metastatic melanoma cells to assume a neural crest cell-like phenotype in an embryonic microenvironment, *Proc Natl Acad Sci USA*, vol. 103(10), pag. 3752-3757.

Livraghi T, Ceriani R, Palmisano A, Pedicini V, Pich MG, Tommasini MA, Torzilli G, 2011, Complete response in 5 out of 38 patients with advanced hepatocellular carcinoma treated with stem cell differentiation stage factors: case reports from a single centre, *Curr Pharm Biotechnol*, vol. 12(2), pag. 254-260.

Livraghi, T, Meloni F, Frosi A, Lazzaroni S, Bizzarri M, Frati L, Biava PM, 2005, Treatment with stem cell differentiation stage factors of intermediate-advanced hepatocellular carcinoma: An open randomized clinical trial. *Oncol Res*, 15(7-8): 399-408.

Malvezzi M, Arfe A, Bertuccio P, Levi F, La Vecchia C, Negri E, 2011, European cancer mortality predictions for the year, *Ann Oncol*, vol. 22, pag. 947–956.

Nishino M, Jackman DM, Hatabu H, Yeap BY, Ciof-fredi LA, Yap JT, Jänne PA, Johnson BE, Van den Abbeele AD, 2010, New Response Evaluation Criteria in Solid Tumors (RECIST) guidelines for advanced non-small cell lung cancer: comparison with original RECIST and impact on assessment of tumor response to targeted therapy. *AJR Am J Roentgenol*, vol. 195(3), pag. W221-228.

Peeters Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, André T, Chan E, Lordick F, et al., 2010, Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol* vol. 28, pag. 4706–4713.

Pierce GB, Wallace C, 1971, Differentiation of malignant to benign cells, *Cancer Res*, 31, pag. 127-134.

Sodergren SC, White A, Efficace F, Sprangers M, Fitzsimmons D, Bottomley A, Johnson CD, 2014, Systematic review of the side effects associated with tyrosine kinase inhibitors used in the treatment of gastrointestinal stromal tumors

on behalf of the EORTC Quality of Life Group. *Crit Rev Oncol Hematol*, vol. 91, pag. 35–46.

Telerman A, Amson R, Hendrix MJ, 2010, Tumor reversion holds promise. *Oncotarget*, vol. 1, pag. 233-234.

Telerman A, Amson R, 2009, The molecular programme of tumour reversion: the steps beyond malignant transformation. *Nat Rev Cancer*, vol. 9, pag. 206-216.

Topczewska, JM, Postovit LM, Margaryan NV Sam A, Hess AR, Wheaton WW, Nickoloff BJ, Topczewski J, Hendrix MJ, 2006, Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat Med*, vol. 12(8), pag. 925-932.

Van Cutsem E, Nordlinger B, Cervantes A, 2010, Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. *Ann Oncol*, vol. 21(5), pag. 93–97.

Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, Canon JL, Van Laethem JL, Maurel J, et al., 2007, Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemo-therapy-refractory metastatic colorectal cancer. *J Clin Oncol*, vol. 25, pag. 1658–1664.

Weinberg RA, 2014, Coming full circle-from endless complexity to simplicity and back again, *Cell*, vol. 157, pag. 267-271.

Wheatley D, 2014, Cancer research: quo vadis—to war? *Ecancermedicalscience*, vol. 8, pag. ed45.

Wise PH, 2016, Cancer Drugs, Survival, Ethics. *BMJ*, vol. 355, pag. 1-3.