INFLA-CARE

PROJECT FINAL REPORT

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Final publishable summary report

Epidemiological and experimental evidence supports a link between chronic inflammation and cancer and indicates a role for inflammatory cells in the initiation, progression and metastasis of malignancy. The collaborative integrated project INFLA-CARE has established a European network of scientific and technological excellence in the field of 'Inflammation & Cancer' which capitalises on the available expertise with a view to develop effective anti-inflammatory strategies and novel agents or screening platforms for cancer prevention and treatment.

The project has specifically sought to identify molecular and cellular targets for cancer therapy through the development and systematic study of state-of-the-art pre-clinical models of inflammation-driven cancer. During the period of the grant, INFLA-CARE researchers have developed and utilised a variety of *in vitro* and *in vivo* models to study the role of cytokines and pro-inflammatory signalling mediators in lung, liver and colorectal carcinogenesis and analyse the molecular pathways involved, including the NF-κB and MAPK cascades. The consortium has also discovered novel links between ageing, obesity and inflammation which may impact on carcinogenesis.

By mobilising the outstanding research experience and technological capacities of the network participants, the program has identified and tested new diagnostic and therapeutic strategies which are expected to lead to improved detection, prevention and management of several types of human cancer. It has also discovered novel interactions between the immune system and the response to chemotherapy which will enrich the oncological armamentarium towards 'patient-tailored' therapies. These studies have overall yielded significant results which provided the basis for the rational design of novel therapeutic strategies. Procedures and platforms have also been established for the analysis and further evaluation of data that have been generated, including bio-computing software for data integration and concept discovery.

In addition to its major scientific contributions, INFLA-CARE has worked to ensure spreading of scientific excellence and dissemination of knowledge beyond the network, by encouraging innovation and transfer of knowledge and by raising public understanding of scientific and health issues.

Summary description of project context and objectives

Dunn and colleagues proposed in 2004[1] that the relationship between the immune response and tumorigenesis is characterized by 3 stages, collectively called immunoediting. The first stage, termed *elimination*, represents the period in which the immune system, through successful immunosurveillance, destroys precancerous and cancerous cells. In *equilibrium*, the second stage, cancer cells have begun to develop capabilities to overcome immunosurveillance but the balance between "immune patrol" and tumorigenesis is still preserved. In the third stage, named *escape*, the cancer cells manage to override the immune response, resulting in aberrant cell proliferation and tumor development.

The significance of immunoediting in carcinogenesis is highlighted by a seminal paper published in ***Science***[2] by a team of scientists that includes INFLA-CARE researchers, showing that chromosomal content in a tumor is controlled indirectly by an immunosurveillance mechanism which ensures the elimination of hyperploid cells. In particular, this work demonstrated that hyperploid cells become immunogenic because of a constitutive endoplasmic reticulum stress response resulting in the aberrant cell surface exposure of calreticulin. CRT facilitates the phagocytosis of stressed and dying cells by macrophages as well as by antigen-presenting dendritic cells and it thus part of a barrier mechanism to restrain tumor growth [2].

On the other hand, the powerful impact of immune cells on cancer has been recognised for many years. Epidemiological studies indicate that approximately 15% of all malignancies can be attributed to infectious agents, such as viruses and bacteria that are able to evade clearance by the immune system and establish a state of chronic inflammatory imbalance. Chronic inflammation has, however, a much broader role in the pathogenesis of cancer. Indeed, experimental and clinical evidence suggests that sustained (and frequently sub-clinical) inflammation triggered by chronic exposure to toxic agents, irritants, oncogenes or autoimmune reactions may contribute to the initiation, progression and metastasis of diverse types of human cancer, including lung, colon and breast.

Inflammatory bowel disease is probably the best-known chronic inflammatory disorder which pre-disposes to cancer and includes Crohn's disease and ulcerative colitis. Tumorigenesis in colitis-associated cancer (CAC) is thought to evolve via heterotypic interactions between cancer cells, bacteria flora and multiple recruited or resident stromal cell types forming the tumor microenvironment. A stromal component of particular importance to tumorigenesis, especially in CAC, has been the inflammatory component, which appears to promote the neoplastic potential of intestinal epithelial cells (IECs) via the production of proinflammatory cytokines and chemokines, proangiogenic and growth factors, ROS, and proinvasive matrix-degrading proteases (reviewed in ***Nature***[3] with the acknowledgment of INFLA-CARE support). However, the specific signaling pathways underlying inflammatory cell recruitment and their effector functions during tumorigenesis are not fully understood and relatively little is known about the molecular mechanisms mediating resident stromal cell activation and crosstalk with the adjacent tumor epithelium and its microenvironment.

Hepatocellular carcinoma (HCC) is another disease largely attributed to chronic inflammation. HCC is rapidly increasing globally and is now the 4th major cancer killer in men. Present treatments are limited and prognosis upon diagnosis is bleak unless the patient is suitable for an available liver

transplantation. There is growing evidence for a disease axis involving interplay between inflammatory, fibrogenic and tumourogenic processes in the diseased liver. The links between liver inflammation and fibrosis are well established and at least 90% of cases of hepatocellular carcinoma develop on the background of severe fibrosis. However, mechanisms are less well established although there is growing evidence that the main fibrogenic cell of the liver, the hepatic stellate cell-derived myofibroblast, contributes to tumour growth by generating a microenvironment that is favourable to tumour growth and survival. In particular the hepatic myofibroblast contributes stable collagen-rich extracellular matrix and a soup of pro-inflammatory molecules that act as growth factors for tumour cells.

Lung cancer is the most common cause of cancer-related deaths in the western world. The recent EUROCARE 3 study has showed that more than 230,000 people in the EU died of lung cancer in 2000, that is one in five of all deaths from cancer. A major risk factor for this type of malignancy is the excessive exposure to chemical carcinogens and particles in tobacco smoke which cause genomic instability and low level, often subclinical, chronic inflammation[4,5]. There is also an established link between chronic inflammation caused by silica (silicosis) or bacterial infections and lung carcinoma[6].

Current treatments for cancer include primary tumour resection and/or aggressive radiotherapy and chemotherapy to achieve both local control and therapy for distant metastases. Conventional cancer treatments are therefore designed to target malignant cells. Tumors, however, frequently develop resistance to therapy as a consequence of the multiple genetic and epigenetic changes they accumulate. Drug resistance represents a major clinical problem and cause of mortality among cancer patients and largely accounts for the limited success of the conventional anti-cancer strategies. *Targeting the inflammatory, <u>non-transformed</u> component of tumors may therefore offer an attractive alternative for both the prevention and treatment of malignant disease.*

This **alternative concept** for combating cancer represents the core of the research and technological activities of the collaborative integrated project INFLA-CARE. Through the mobilisation of the research expertise and technological capacities of the INFLA-CARE network participants, we have sought to translate the knowledge of the molecular pathways regulating inflammation-driven cancer into new diagnostic and therapeutic strategies which will be used for the detection, prevention and improved management of several types of human cancer.

The development of such novel approaches to fight cancer has been so far impeded by the near absence of concerted trans-disciplinary collaborative efforts which could rigorously define the influence of the inflammatory microenvironment on tumor growth and, in particular, identify the early molecular events that trigger tumor initiation in chronic inflammatory sites. Technological limitations have also precluded the use of microarray-based assays for the retrospective, large scale evaluation of the inflammatory gene expression profile in archived (typically paraffin-embedded) human pre-malignant and malignant tissue specimens, which could lead to the identification of novel disease biomarkers. The development of pre-clinical models which faithfully mimic disease progression in humans and could stimulate novel diagnostic and therapeutic strategies represents an additional major challenge in the field.

INFLA-CARE collectively possesses the technological capability and proven expertise in disease modelling and tissue analysis to **overcome these obstacles** and accelerate the translation of

knowledge obtained by basic research into novel therapies. Our translational efforts were channelled towards the normalisation of the inflammatory network in cancer-prone tissue prior to the appearance of advanced malignant disease. Combination therapies targeting both the inflammatory microenvironment and the tumor cells have also been evaluated, including conventional cancer therapeutics used in the oncological armamentarium. These strategies will be used in a manner that takes into consideration the molecular information about inflammatory components and signal transduction pathways operating in a tumor type-specific manner.

To address the role of chronic inflammation in cancer, INFLA-CARE pursued the following objectives:

1. To characterize the inflammatory cell components and soluble mediators in inflammation-associated carcinogenesis and to define the early molecular events that trigger tumor initiation in chronically inflamed sites.

2. To define molecular mechanisms and signalling pathways by which inflammation and immunity affect cancer development and progression, with emphasis on NF-κB and MAPK modules, and of the post-transcriptional intracellular network in dictating the nature of the inflammatory microenvironment.

3. To develop novel diagnostic and therapeutic anti-cancer strategies based on the improved understanding of the inflammation-cancer link.

4. Aided by 'systems biology' and 'text mining' platforms, to integrate research results into meaningful disease profiles which is likely to lead to new scientific and translational hypotheses.
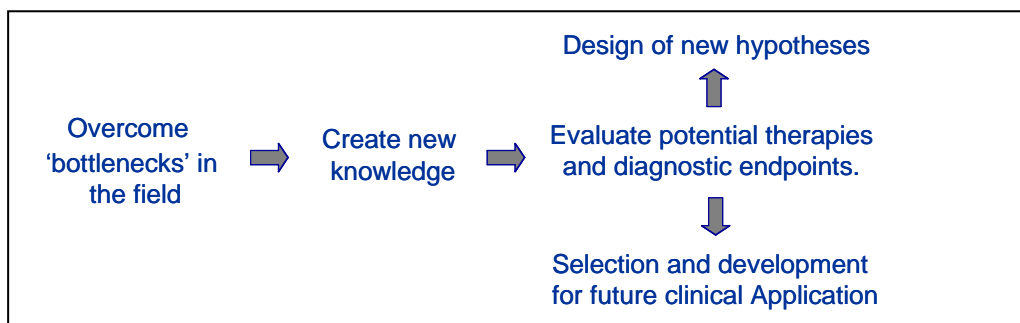
Description of the main S&T results/foregrounds

Hepatocellular carcinoma

We have generated important new data defining the mechanisms that regulate hepatitis, liver ageing, fibrogenesis, regeneration and development of HCC. We have developed two new models of HCC that more closely resemble the pathology of human liver disease and that provide tools for new translational science.

The first model developed by the team of Derek Mann (P15) involved adaption of a 40-week diethylnitrosamine (DEN) model of HCC which adult mice are resistant. The adaption was to include
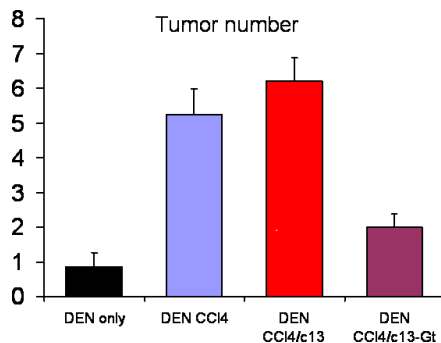

Figure 1. DEN+CCl4 model of HCC

a transitory 12-week period of fibrogenesis induced by repeated (twice weekly) IP administration of carbon tetrachloride (CCl_4) half-way through the 40 week model. The data shown in Fig 1 demonstrate that the CCl_4 injury increases tumour numbers 5-fold. To determine the functions of myofibroblasts in this model we included two further groups of mice in which we followed each CCl_4 injection 24hrs later with administration of naked single chain antibody (ScAB) C1-3 (CCl_4/c13) or C1-3 conjugated to the NF-κB inhibitor and pro-apoptotic compound gliotoxin (CCl_4/c13-Gt). C1-3 is a ScAb that selectively targets liver myofibroblasts due to its specificity for synaptophysin which is not expressed by other cell types in the liver. Similar numbers of tumours between the groups DEN/ CCl_4 and DEN/ CCl_4/C1-3 confirms the tumour promoting effects of fibrogenesis. Reduction of tumour numbers to close that in the DEN-only group in mice treated with C1-3-gliotoxin (DEN/ CCl_4/C13-Gt) provides evidence that myofibroblasts are important non-parenchymal stimulators of HCC growth in the diseased liver.

The second model was discovered by ageing *nfkb1-/-* mice which has a low-grade chronic systemic inflammatory state caused at least in-part by absence of p50:p50 inflammatory repressors. In work now *submitted for publication* (Jurk et al, 2013), P15 have reported inflammation-induced replicative senescence of epithelial cells in these mice, which reduces their life-span to just 20-months. At 20 months all males have evidence of severe spontaneous liver disease inclusive of inflammation, fibrosis and HCC.
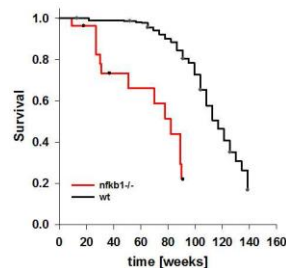

Figure 2. Accelerated aging of nf-kb1-/- mice.

In addition to developing spontaneous age-related liver disease and HCC, the *nfkb1-/-* mouse is highly susceptible to HCC induced by neonatal administration of DEN. In this model wt mice develop an average of 15 tumours on the surface of the liver at week 40. By contrast, the *nfkb1-/-* mouse has an average of 30 tumours at 30 weeks and in excess of 60 tumours by week 40. We have discovered that DEN-injured *nfkb1-/-* mice characteristically have high numbers of neutrophils in their liver associated with increased oxidative stress and genotoxic damage and results from the over-expression of a chemokine network (S100A8/S100A9/CXCL-1/CXCL-2) by *nfkb1*-null damaged hepatocytes. As a consequence, activated neutrophils are continually recruited in high numbers to the

nfkb1-/- liver where they release a number of powerful bioactive molecules that cause collateral cellular and genotoxic damage that promotes tumour growth. As neutrophils are also a feature of human liver disease and HCC, our data are important since they highlight the potential of targeting neutrophils to prevent age-, inflammation- and toxin-induced HCC. This work has been ***submitted for publication*** (Wilson et al, 2013) with the acknowledgment of INFLA-CARE support.

Mice lacking the multidrug resistance-2 gene (*Mdr2-/-*) develop chronic periductular inflammation and cholestatic liver disease resulting in the development of hepatocellular carcinoma (HCC). To address the role of IKK2-mediated NF-κB activation in hepatocytes in the pathogenesis of liver disease and HCC in *Mdr2(-/-)* mice, the team of Manolis Pasparakis (P13) generated Mdr2-deficient animals lacking IKK2 specifically in hepatocytes using the Cre-loxP system. As they have reported in ***PLOS One***[7], IKK2-mediated signaling in hepatocytes protects the liver from damage under conditions of chronic inflammatory cholestasis and prevents the development of severe fibrosis and liver failure. This effect is attributed to the IKK-mediated induction of anti-apoptotic genes in hepatocytes and highlights the multifaceted properties of NF-κB signaling which can promote or inhibit tumorigenesis in a tissue-specific manner.

The work on the *nfkb1* and IKK2 genes made us aware of the close association of classic NF-κB activation by IKK2 with the activation of ERK signalling, this was particularly interesting as ERK-regulated pathways lead to transcriptional stimulation of many fibrosis- and tumour-regulators. In resting cells the MAP3K Tpl2 (Cot) is sequestered in an inactive complex with the *nfkb1* protein p105. In response to IKK2 phosphorylation the p105 protein is processed to its shorter p50 form and this leads to release and subsequent activation of Tpl2. The Tpl2 kinase triggers a cascade of phosphorylation events leading to the phosphorylation and activation of ERK1/2. Cells lacking *nfkb1* have low levels of Tpl2 due to the kinase being unstable in the absence of p105. Hence we were interested to determine if Tpl2 is a regulator of fibrogenesis given that we had previously shown nfkb1-/- mice are susceptible to liver fibrosis. In work recently published in ***Hepatology***[8], the team of Derek Mann reported that Tpl2 is expressed in liver macrophages and myofibroblasts and is required for TLR-induction of the pro-fibrogenic tissue inhibitor of metalloproteinases 1 gene (TIMP-1). They additionally discovered that *tpl2-/-* mice develop less liver fibrosis than wt mice in two distinct models of liver injury, CCl4 and the MCD diet model of steatosis-induced fibrosis.

In parallel, the **Eliopoulos team (P1)** investigated the role of Tpl2 in the acute model of ConA-induced T cell-mediated hepatitis. They have found that *tpl2-/-* mice are partially resistant to conA-induced liver damage and that administration of a TPL2 kinase inhibitor ameliorates hepatitis. Primed by these findings, they analysed the impact of TPL2 on various inflammatory cells which may contribute to this pathology and found that Tpl2 is required to activate NKT cells which produce pathogenic levels of inflammatory cytokines responsible for the phenotype. Mechanistic studies showed that TPL2 regulates ERK in this model which in turn leads to stimulation of IL-4 and IFNγ secretion by NKT cells. Progress in this project was greatly facilitated through a visit of an **Eliopoulos**' team member (Dimitra Vyrla) to the lab of **Derek Mann** (P15) and a joint paper is ***in preparation*** (Vyrla et al, 2013). Therefore, TPL2 is a pro-fibrogenic and pro-inflammatory kinase in the liver and a putative therapeutic target.

Unlike the effects of TPL2 and Raf on liver pathology, the team of **Josef Penninger (P2)** found that JNK signaling which is implicated in cytokine signaling and inflammation, does not have a major role in hepatitis in the mouse. They developed conditional knock-out mice with specific ablation of MKK7 in the liver (MKK7fl/fl alfp-Cre) and hemopoietic compartment (MKK7fl/fl Mx1-Cre) which were exposed to 2 models of hepatitis: ConA and LPS/Gal/N. Neither hepatocyte nor hemopoietic ablation of MKK7 affected overall survival and hepatic damage.

Like TPL2, Raf is a MAP3 kinase with an important role in the regulation of ERK signaling. However, whereas TPL2 is important in the context of inflammatory signaling Raf predominantly transduces growth factor – mediated ERK signals. In the context of INFLACARE, the team of **Manuela Baccarini (P17)** investigates the effects of B+CRaf ablation on inflammation and inflammation-driven tumorigenesis in the liver. They have found that the concomitant ablation of both B and CRaf did not give rise to spontaneous inflammation; in addition, the phenotype of the compound knock-out was identical to that obtained by the ablation of C-Raf only; therefore, they proceeded to investigate in depth the effect of C-Raf ablation on chemically induced hepatocarcinogenesis. Contrary to its essential protumorigenic role in Ras-driven epidermal [9] and lung [10,11] tumorigenesis, C-Raf actually has a cell autonomous tumor suppressor role in hepatocellular carcinoma.
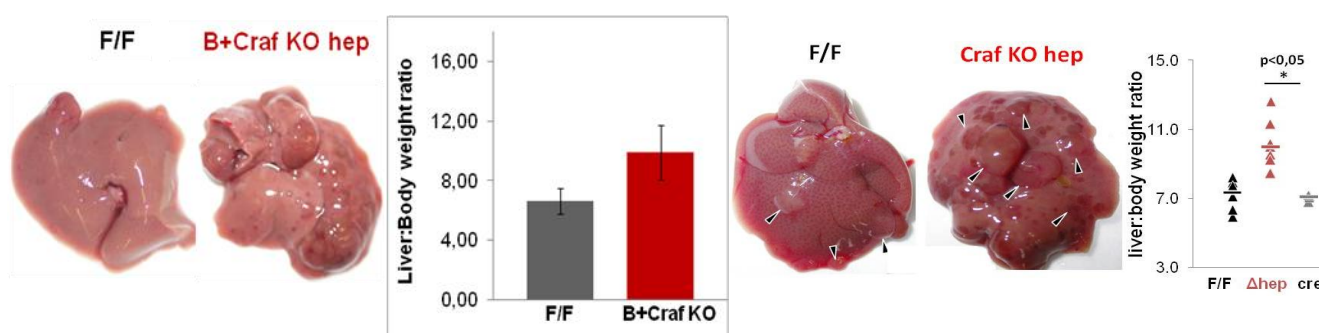

Figure 4 – Hepatocyte-restricted compound B+CRaf ablation and CRaf ablation increase tumor load in a chemical model of hepatocarcinogenesis. The plots show the increase in liver/body weight ratio F/F, B+CRaf and CRaf KO[hep] mice 30 weeks after induction of hepatocarcinogenesis by dietyhlnitrosamine/phenobarbital treatment. The KO samples are shown in red.

Characterization of the disease has shown that CRaf KO[hep] livers contain large numbers of hematopoietic cells, particularly macrophages. This is important because the chemical carcinogenesis protocol critically depends on the interaction between hepatocytes and macrophages, which produce cytokines supporting tumor development [12]. In line with this, and in good correlation with the larger numbers of macrophages observed, higher levels of MCP-1, MCP-3, CXCL1, IL-6, TNFα, and IL-1β were detected in the livers of CRaf KO[hep] mice, suggesting a role for CRaf in controlling hepatocytes-derived inflammatory signals.

To determine whether CRaf had a similar role in macrophages, the team ablated CRaf in parenchymal and nonparenchymal cells before initiation of tumorigenesis using the inducible MxCre recombinases (CRaf KO[p/np]); this abrogated both the increase in macrophage numbers observed in the CRaf KO[hep] mice and the increase in tumor load, indicating that a) this CRaf KO[hep] phenotype depends on the interplay between hepatocytes and macrophages; and b), that the C-Raf-deficient

macrophages cannot sustain it. An in-depth investigation of these cells has shown that they proliferate and produce chemokines/cytokines to an extent similar to the wild-type macrophages; however, like other C-Raf-deficient cells [13], they are unable to migrate and infiltrate the liver *(Jeric at el., in preparation)*. We therefore conclude that CRaf plays a cell-autonomous tumor suppressor role in hepatocytes, and, being essential for macrophage migration, a crucial role in creating a tumor-promoting environment. The mechanism behind the tumor suppressor role is still under investigation but appears to rely on the control of protein turnover, which in turn affects the activation of three major protumorigenic pathways *(Cavallo et al., in preparation)*.
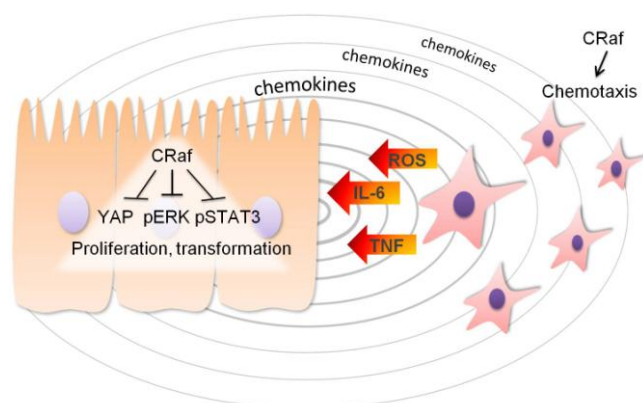

Figure 5 – Tumor suppressor and tumor promoting roles of CRaf in liver carcinogenesis. See text for details.

Up-regulation of ribosome biogenesis is largely responsible for the increase in the protein synthesis that characterizes malignant tumours. The **Volarevic team (16)** hypothesized that this pathway may serve as a major barrier to tumorigenesis and that it may be manipulated therapeutically for the treatment of cancer. To manipulate ribosome biogenesis in vivo, they developed a conditional model for deleting the RPS6 ribosomal protein gene in the mouse and showed that ribosomal protein RPS6-haploinsufficiency limits ribosome biogenesis and consequently triggers a p53-dependent checkpoint in highly proliferative embryonic cells.

In this project, they have developed primers for RT-PCR array covering a panel of 80 RP genes and as well as 47S pre-rRNA and tRNA and 7SL. Using these RT-PCR arrays they interrogated the transcriptome for time-dependent changes in the expression of RP as well as Pol I and Pol III genes in the mouse model of DEN-induced inflammation-driven hepatocellular carcinoma (HCC) at 2, 4, 6, 8, 10, 12 and 14 months. RPS15a, RPL35, RPL11, RPL26 and RPL36 mRNAs were dramatically up-regulated at month 14 (between 1.7- and 3.5-fold) whereas the expression of RPS2, RPS26, RPS27, RPLP0, RPS15a, RPL37, RPS23 and RPL5 mRNAs was specifically down-regulated. The expression of the 47S rRNA showed minimal changes after 2, 4 and 6 months of DEN/PB exposure, but at month 10, when first tumors become macroscopically visible, it was significantly increased (1.6-fold). Surprisingly, there was no significant change in the level of 47S pre-rRNA at month 14. Further research will be required to understand the precise role of these alterations in the pathogenesis of HCC. Additionally, the effects of c-Myc on the expression of all RPs using ribosome biogenesis RT-PCR array were evaluated in mouse embryonic fibroblasts in collaboration with the Oren team and a paper acknowledging INFLA-CARE was published in *Molecular Cell* (2012)[14]. These human and mouse ribosome biogenesis RT-PCR array can be used to analyse ribosome biogenesis in physiological and various pathological conditions.

The team also characterized the molecular mechanisms by which impaired ribosome biogenesis leads to activation of a p53-dependent checkpoint. In particular, they have analyzed p53 signaling following depletion of RPS6 or exposure to other types of ribosomal stress in cancer cell lines and found that RPL5 and RPL11 are the key positive regulators of the p53 tumor suppressor in response to RPS6 deficiency as well as various other ribosomal biogenesis stressors, such as the chemotherapeutic agents 5-FU and actinomycin D. The results on p53 activation upon impairment of ribosome biogenesis are briefly summarized below and depicted in Figure 6. Under unstressed conditions, newly synthesized RPs of 40S and 60S ribosomal subunits are imported into the nucleolus. Upon impairment of ribosome biogenesis, the majority of RPs are synthesized, but they are degraded by nuclear 20S proteasomes. In contrast, RPL5 and RPL11 are not degraded, and they accumulate in the nonribosomal fraction, where they bind Mdm2. RPL5 and RPL11 colocalize with Mdm2, p53, and PML in the nucleolus after impairment of ribosomal biogenesis, where full p53 activation probably takes place. Less efficient import of newly synthesized RPL5 and RPL11 into the nucleolus upon inhibition of ribosomal biogenesis may also contribute to their accumulation in the nonribosomal fraction. In the absence of RPL5, ribosome-free RPL11 is degraded by proteasomes upon ribosomal biogenesis stress, suggesting that RPL5 and RPL11 protect each other from degradation and explaining their mutual requirement in p53 activation. This work has been published in the ***Proceedings of the National Academy of Sciences USA*** with the acknowledgment of INFLA-CARE support[15].
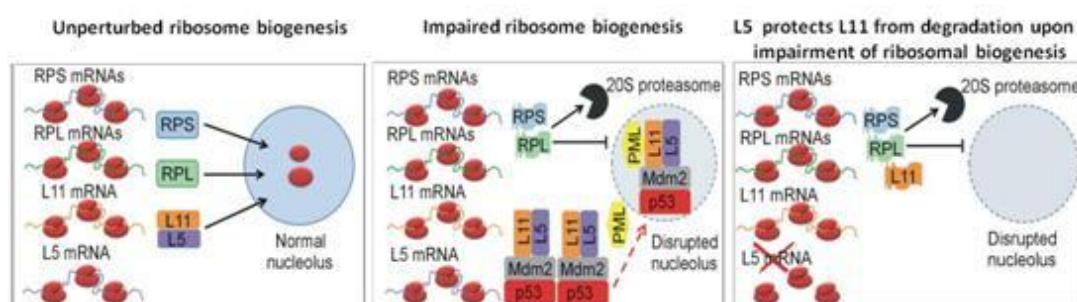
Fig. 6. *Model explaining the role RPL5 and RPL11 in p53 activation upon impairments of ribosome biogenesis.*

Finally, the Volarevic team analysed the effects of RPS6-heterozygosity in inflammation-driven hepatocellular carcinoma. They have found that deletion of one RPS6 allele inhibits HCC growth (Fig. 7). p53 was up-regulated at 2 days after deletion of one RPS6 allele in the liver of Mx-CRE+/S6wt/flox;p53flox/flox mice. To assess the contribution of a p53-dependent checkpoint to anti-HCC effects following deletion of one RPS6 allele in DEN/PB- induced HCC, the Volarevic team decided to co-delete one RPS6 allele together with the floxed p53 alleles. Mx-CRE+/S6wt/flox;p53flox/flox and Mx-CRE-/S6wt/flox ;p53flox/flox mice were subjected to DEN/PB exposure. Unfortunately, the majority of Mx-CRE+/S6wt/flox; p53flox/flox mice showed the enhanced susceptibility to various cancers and they died before month 12 (data not shown). This is probably due to a background CRE-mediated deletion of p53, the key tumor suppressor in mammalian cells, in combination with cancerogenic effects of DEN. Thus, the contribution of p53 to anti-HCC effects following deletion of one RPS6 allele could not be assessed in this model and instead the team evaluated the effects of pifithrin-alpha, which is a p53 inhibitor, on the anti-HCC potency of deletion of RPS6 allele. DEN/PB-treated Mx-CRE+/S6wt/flox; p53flox/flox and Mx-CRE-/S6wt/flox;p53flox/flox mice were injected with poly(I-C) at month 14. These mice were

treated daily with pifithrin-alpha for one month after poly(I-C) injection. Pathohistological evaluations of liver sections at month 15 will be completed by October 2013 and a paper is expected to **be submitted for publication** in 2014.
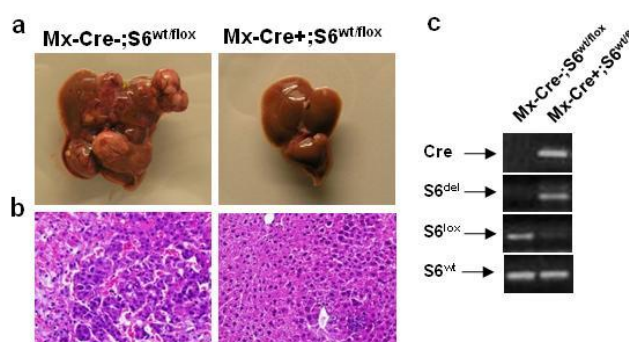

Fig. 7. *Deletion of one RPS6 allele inhibits HCC growth. (a) Large tumors were clearly visible on the surface of livers from Mx-CRE-/RPS6wt/flox but not Mx-CRE+/RPS6wt/flox mice one month after poly(I-C) treatment. (b) Liver sections of Mx-CRE+/RPS6wt/flox mice showing normal histology. (c) PCR analysis of RPS6 flox and RPS6 del alleles.*

Lung and breast cancer

The vast majority of pre-neoplastic lesions such as hyperplasia of the lung and breast are quiescent and do not progress to form overt tumors. It has been proposed that oncogenic stress activates the DNA damage response (DDR) and the key tumor suppressor p53, which prohibits tumor growth. However, the molecular pathways by which cells sense a premalignant state *in vivo* is largely elusive. The teams of Ari Eliopoulos (Co-ordinator, P1), Josef Penninger (P2), Kontoyiannis and Panayotou (P4) have identified novel regulatory pathways in lung and breast carcinogenesis.

To genetically assess the role of JNK signaling during lung tumorigenesis, the group of **J. Penninger** created and analysed MKK7 floxed mice. The stress signaling kinase MKK7 is a specific and essential bottleneck within the JNK signaling cascade. P2 established conditional knock-out models for KRas-driven non-small-lung-cancer (NSLC) as well as NeuT-driven mammary cancer. Tissue-specific inactivation of the stress signaling kinase MKK7 in KRas^{G12D}-driven lung carcinomas as well as in NeuT-driven mammary tumors markedly accelerated tumor onset and reduces overall survival. Mechanistically, MKK7 acts via JNK1/JNK2 and this signaling pathway directly couples oncogenic and genotoxic stress to p53 stability required for cell cycle arrest and suppression of epithelial cancers. These results for the first time showed that MKK7 functions as a major tumor suppressor for lung and mammary cancer in mouse and identified MKK7 as a vital molecular sensor to set a cellular anti-cancer barrier. This work was published in *Nature Genetics* [16] and performed in collaboration with the InflaCare network member V. Gorgoulis (P11).

JNK is not only positively regulated by upstream kinases such as MKK7 but is also subject to negative regulation by the action of dual-specificity phosphatases (DUSP). The **Panayotou team** has focused on a relatively less understood member of this family, DUSP8 or M3/6, which specifically dephosphorylates and regulates the activity of the JNK MAPK and interestingly, at the same time is itself phosphorylated by JNK upon stress stimulation. The DUSP8 phosphatase is primarily expressed in lung, brain and heart and it is of particular interest due to the complex role of JNK in tumorigenesis. We have performed an extensive characterization of the DUSP8 protein by identifying and mutating key JNK-phosphorylation sites on the phosphatase and assaying the resulting DUSP8 mutants for enzymatic activity, JNK binding, stability, localization and effect on JNK activation. We have shown that phosphorylation of DUSP8 in response to stress stimuli results

in the enhancement of JNK activation and is therefore a mechanism for regulating the cellular response to stress. We have also examined in detail the specific interaction of DUSP8 with the different members of the JNK family and shown that DUSP8 preferentially binds to and dephosphorylates the JNK1β and JNK2α isoforms of the family. This is especially interesting given the distinct differential expression or activation of the different isoforms in various systems, suggesting individual roles for each isoform. Furthermore, the Panayotou team has demonstrated that DUSP8 binds differentially to members of the JIP scaffold family, as we have shown its interaction to be constitutive with JIP1 and JIP2 while stimulus-dependent with JIP3 – a scaffold protein preferentially expressed in the nervous system. Attempts to generate a knock-in mouse with a catalytically inactive DUSP8 were unsuccessful; however, *in vivo* work performed with arsenite-treated mice in order to detect proteomic changes in the lung, resulted in the identification of 144 differentially expressed proteins in mice exposed to arsenite compared to saline controls, including stress-regulated proteins and proteins involved in metabolic processes. These data have been published in ***Cell Signaling*** (2013)[17] and additional manuscript has been submitted for publication.

The **Eliopoulos team** has found that the Tpl2 kinase[18] functions as a tumor suppressor of urethane-induced lung cancer in the mouse (WP5). The team also demonstrated for the first time that Tpl2 expression is downregulated in human lung tumors as a result of multiple genetic and epigenetic aberrations, including loss of heterozygocity at the Tpl2 gene locus, miR-370 upregulation affecting Tpl2 transcripts and activation of oncogenic Ras signalling, and that low Tpl2 levels correlate with reduced lung cancer patient survival (Fig. 8). Tpl2 was also found to regulate MKK7/JNK signalling thereby controlling the p53 tumor suppressor pathway *in vitro* and *in vivo* and the induction of apoptosis, a barrier to cancer. In addition to their scientific impact, these findings have the potential of developing markers that could be useful for determining lung cancer patient prognosis. This work was recently published in the ***Proceedings of the National Academy of Sciences USA*** [19] with the acknowledgment of INFLA-CARE support and performed in collaboration with the InflaCare network member histopathologist V. Gorgoulis (P11).
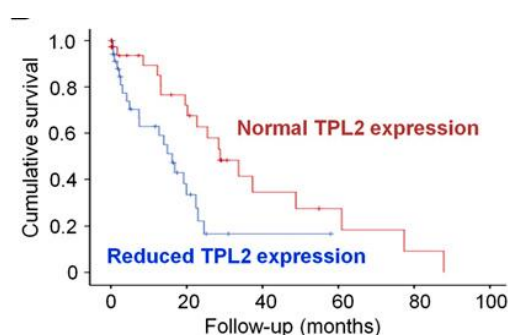
Fig. 8: Reduced TPL2 mRNA levels correlate with poor lung cancer patient prognosis.

In follow-up studies, the team has also revealed a novel mechanistic link between Tpl2 and p53. They have found that a fraction of Tpl2 resides in the nucleolus where it physically associates with nucleophosmin (NPM) and affects its release to the nucleoplasm following nucleolar stress and DNA damage. NPM is a negative regulator of Mdm2:p53 interactions and knock down of Tpl2 attenuated the translocation of NPM from the nucleoli to nucleoplasm, reduced the binding of NPM to Mdm2 and resulted in reduced levels of p53 accumulation in stressed cells. This work was performed in collaboration with the team of Sinisa Volarevic (P16) and a **manuscript acknowledging INFLA-CARE support is in preparation.**

The Kontoyiannis Lab (P4) aimed to assess the importance of the post-transcriptional regulators HuR and AUF1 in chronic inflammation and cancer by using obligatory and tissue restricted mutations for these RNA-binding proteins (RBPs) (WP6). During the early phases of the project they identified that the genetic loss of the RBP HuR arrested the branching morphogenesis stage of lung

development, due to defects in mesenchymal:epithelial interactions due to the loss of the key inducer of outgrowth and epithelial branching, FGF10, which is also involved in tissue remodelling during cancer formation. HuR was found to bind to the Fgf10 and Tbx4 mRNAs in the presence of mesenchymal regulators such as retinoic acid or FGF9, and as a result its deletion abolished the inducible regulation of FGF10. This initial set of data which were published in ***Developmental Biology*** [20] suggested that HuR is required for the maintenance and expansion of lung progenitor cells and as such it could play a pro-tumorigenic role in lung cancer. However, restricted deletion of HuR in lung epithelia supported a clear anti-tumorigenic function: mutant mice developed a sporadic yet spontaneous phenotype consisting of increased inflammation, emphysema, regional bronchiolar and alveolar hyperplasia, and late tumour development. In addition, these mice appeared heavily susceptible to urethane- and mutant KRas-induced lung carcinogenesis; conversely, the overexpresion of HuR attenuated the development of these inducible cancers. Mechanistically, the anti-tumorigenic effects of HuR related to its regulatory effects upon mRNAs controlling the renewal and differentiation of epithelial lung progenitors. In contrast to HuR, the RBP AUF1 did not appear to be involved neither in lung physiology nor tumorigenesis; this suggests that AUF1 does not have any clear antagonistic functions to HuR but is rather involved in distinct events of RNA regulation. This work, representing deliverable 6.6 has been ***submitted for publication***.

Prompted by findings that highlight an important role for IL-1α in supporting angiogenesis, the group of Ron Apte (P3) has investigated the impact of IL-1β in the control of antitumor immunity versus progression in a transplantation model of experimental lung metastasis, using colon, lung and melanoma tumor cells. Their results were published in 2 papers the ***Journal of Immunology*** (2011)[21,22] and demonstrate that the absence of IL-1 signaling or its excess in the lung microenvironment in IL-1β and IL-1R antagonist knockout [KO] mice, respectively, result in poor prognosis and reduced T cell activity, compared with WT mice. In IL-1β KO mice, enhanced Treg cell development/function, due to a favorable in situ cytokine network and impairment in APC maturation, results in suppressed antitumor immunity whereas in IL-1R antagonist KO mice, enhanced accumulation and activity of myeloid-derived suppressor cells were found. In the microenvironment of lung tumors, IL-1 induces IL-17 through recruitment of γ/δ T cells and their activation for IL-17 production, with no involvement of Th17 cells. Reduced tumor progression along with improved T cell function were observed in IL-17 KO compared with WT mice. Overall, these results highlight the critical and unique role of IL-1, and cytokines induced by it such as IL-17, in determining the balance between inflammation and antitumor immunity in specific tumor microenvironments. Thus, intervention in IL-1/IL-17 production could be therapeutically used to tilt this balance toward enhanced antitumor immunity.

IL-18 was also shown by the consortium to influence lung metastasis. In 2 papers published in ***Cancer Research***[23,24] with the acknowledgment of INFLA-CARE support, the team of Laurence Zitvogel (P7) reported that IL-18 increases the number of metastases in a B16 melanoma lung metastasis model by controlling the proliferation of the 'immuno-suppressive' NKreg cells. In contrast, NKreg cells do not expand in hosts deficient for the IL-18Ra/MyD88 pathway and less metastases are formed in these animals. Similar results were obtained using IL-18BP which neutralizes the tumor-derived IL-18 and highlights its potential as anti-tumor agent.

Inflammation and cancer in the colon

The consortium has utilized **chemical** (azoxymethane/Dextran Sulfate; AOM/DSS), **genetic** (transgenic mouse model expressing constitutively active IκB kinase 2 in intestinal epithelial cells) and **pathogen-induced** (*H. Hepaticus*) models of colitis and CAC to dissect the role of cytokines, inflammatory cell types and signalling pathways in disease pathogenesis.

The teams of **Eliopoulos (P1), Almac (P19) and Biomedcode (P20)** collaborated in the context of WP3 and generated transcriptional profiles of distal and proximal gut of mice exposed to the AOM/DSS protocol with the aims to: (a) obtain a broad picture of molecular changes occurring during progression from colitis to colon cancer and (b) understand the differential susceptibility of the distal gut which develops cancer and the proximal colon tissue which rarely develops tumors. The results demonstrated that the gene expression differs between proximal and distal colon in control conditions. Following administration of AOM/DSS, there is marked difference in the gene expression profiling between proximal and distal tissue with a progressive and dramatic increase in differentially expressed genes in the latter case. Bioinformatic analyses identified pathways related to inflammatory responses which are over-represented in the disease distal tissue, whereas the proximal gut is characterized by induction of

Figure 9: Heat map of the differentially expressed transcript lists with only common genes between at least two time points.

pathways which relate to tissue morphogenesis and metabolism. A possible link between metabolism and cancer in this model is currently being evaluated by the Eliopoulos' team.

Soluble mediators and immune cells: The transcriptional profiling revealed a number of cytokines to be up-regulated in the distal gut during DSS/AOM-induced CAC. Among them, the functional role of IL-1α, IL-1β, IL-23, IL-18 and IL-15 was studied by the consortium.

The team of **Ron Apte (P3)** has found that IL-1 family genes (IL-1α and IL-1β) play a significant and differential role in induction of colon inflammation. IL-1α KO mice were relatively resistant to the development of colitis, whereas mice deficient in IL-1β were very susceptible to DSS-induced colitis. To further our understanding of mechanisms involved in chronic inflammation, P3 established protocols for isolating lamina propria infiltrating leukocytes and characterizing them by FACS analysis. This work revealed that in mice deficient in IL-1Ra, an increased number of pro-tumorigenic myeloid derived suppressor cells (MDSCs) exists in comparison to other strains of mice. This might explain the early development of pre-cancerous lesions in these mice after termination of DSS-treatment. During their study, P3 have shown that in the colon, IL-1α and IL-1β are expressed by different cell types. Thus, IL-1α is expressed in epithelial cells, but also in some infiltrating myeloid cells, whereas IL-β is solely expressed in myeloid cells. Additional studies showed that mice deficient in IL-1α in colon epithelial cells display a mild form of the disease, whereas
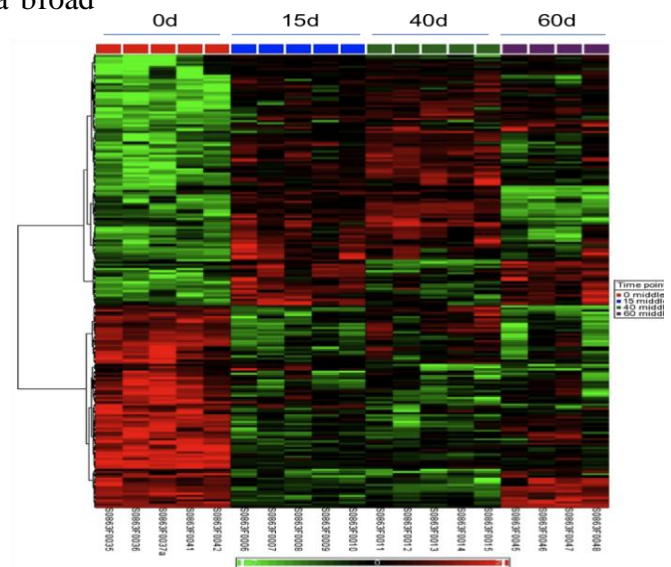
15

deficiency of IL-1α in myeloid cells lead to exacerbation of colitis. Thus, IL-1α expressed from epithelial cells is the main player in both acute and chronic colon inflammation, as well as the development of inflammation related CRC. Further mechanistic studies indicated that IL-1 plays an important role in the interplay between initiation of tissue damage versus repair; the type and amount of IL-1 are critical in these processes. This work has been published in *Gut* (2013)[25] and the *Journal of Immunology* (2013)[26], acknowledging INFLA-CARE support.

IL-15 is a pleiotropic cytokine functioning in both the innate and adaptive immune systems. This cytokine has been recognized to be a promising cytokine for immunotherapy of cancer in particular for its ability to stimulate natural killer and CD8 T cells. The team of **Bulfone-Paus (P9)** in collaboration with histopathologist **V. Gorgoulis (P11)** investigated under WP2 the role of IL-15 and IL-15Rα in inflammation and carcinogenesis using the acute DSS and chronic DSS/AOM mouse models. It was found that IL-15^{-/-} mice display a more severe acute inflammation in the median and distal colon epithelium compared to the WT mice and develop more and bigger tumors compared to the WT mice, suggesting a potent suppressive effect of IL-15 on tumorogenesis. In addition, P9 has generated CD11c-IL15 knock-in mice in which IL-15 expression is confined to CD11c^{+} cells (mostly dendritic cells). The IL-15 expression in these animals impacts on the recovery of CD8 T cells and NK cells and results in the development of less and smaller tumors than IL-15^{-/-} animals treated with AOM/DSS. Therefore, the suppressive effect of IL-15 resides in the immune system and the production of the cytokine by epithelial cells appears dispensable for this inhibitory activity in the DSS/AOM model.

IL-23 is a pivotal player in driving intestinal inflammation. In the context of INFLA-CARE, **Fiona Powrie**'s lab (P8) developed a bacteria-driven colitis-associated cancer (CAC) model using the carcinogen 2-azoxymethane in combination with *H. hepaticus* infection (*Hh*+AOM). Mice were infected with *H. hepaticus (Hh)* and after 6 weeks treated weekly for 5 weeks with AOM. Histopathological analysis showed that mice developed invasive colon carcinoma after 3-5 months, at the sites of highest inflammation in the colon and cecum. In a paper published in *Nature* in 2010 and reviewed in *the same journal* [3] in 2011 they reported the discovery of an innate cell population, ILC (innate lymphoid cells) with lymphoid features, which produces IFN gamma, IL-17 and IL-22 in response to IL-23. These cells are drivers of innate intestinal inflammation in different mouse models including the *Helicobacter hepaticus* model.

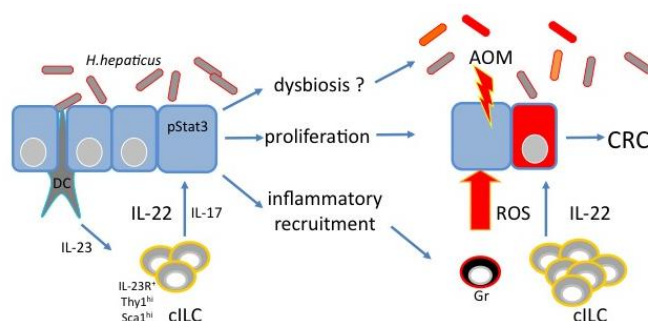**Fig.10: IL-22 sustains inflammation driven-cancer.** Model of IL-22 production by cILC sustaining colon cancer in the *Hh*+AOM model.

They investigated if any of the ILC produced cytokines, IL-17, IL-22 or IFNγ could sustain bacteria-induced cancer. As the heterodimeric IL-22R is predominantly expressed on epithelial cells they hypothesized that IL-22 acting on epithelial cells can initiate a deregulated inflammatory response

and repair programme that can promote CAC. Indeed, functional analysis showed that IL-22 acting on colonic epithelial cells is inducing cell proliferation as measured by increased levels of Ki-67, production of anti-microbial peptides (*RegIIIg, RegIIIb* and *S100A8)* and granulocyte-attracting chemokines *(Cxcl-1)* via Stat3 activation. These results which were published in the ***Journal of Experimental Medicine*** indicate a strong and almost exclusive role for IL-22 in the epithelial-Stat3 axis in this model, regulating epithelial proliferation, myeloid cell recruitment and antimicrobial defence[3] (Fig. 10).

The Powrie team also investigated the presence of IL-22 in human spontaneous CRC in fresh and paraffin embedded tissue. As IBD patients undergo a very tight screening process, no IBD associated cancer samples were available. Interestingly they found that IL-22 could be detected by IHC and IF in human CRC. IL-22 was produced by CD3+ T cells and CD3- cells. Analysis of matched pairs of CRC and adjacent tissue showed a more than 2-fold upregulated expression of IL-22 in tumour versus normal tissue in 58% of matched pairs. The presence of IL-22 producing cells within tumours could point towards a possible therapeutic application of IL-22 blockade in some CRC patients.

Signaling mediators

To study the role of inflammatory signalling pathways in colon cancer, **P13 (M. Pasparakis)** developed a genetic model in which constitutively active IKK2 (IKK2ca^{IEC}) was expressed specifically in intestinal epithelial cells (IEC). In a paper published in the ***Journal of Clinical Investigation*** (2011) [27], they showed that these mice develop intestinal tumors. For studying the role of NF-κB-driven inflammation in intestinal tumourigenesis, P13 crossed the IKK2ca^{IEC} mice with MyD88-deficient animals and found that the double knockouts developed a much more severe phenotype leading to the death of most of the mice at an early age. This is a very interesting finding indicating that TLR signalling might have a protective function in this model of inflammation, which will be followed-up with additional experiments using TLR knockouts and have also plans to address the role of the microbiota using antibiotic and germ-free experiments.

To address the role of TNF, P13 crossed the IKK2ca^{IEC} mice with TNFR1 knockouts and found that TNFR1 deficiency did not prevent the development of inflammation and intestinal tumorigenesis. Also these results are interesting as they indicate that TNFR1 signalling may not be required for the development of intestinal inflammation and cancer driven by epithelial NF-κB activation. A paper describing these findings **is expected to be submitted for publication** during 2014.

Related to this analysis is the observation that mice with IEC-specific knockout of FADD, a central adaptor in death receptor-induced apoptosis, spontaneously develop severe erosive colitis, loss of Paneth cells and enteritis. Using genetic rescue experiments P13 could show that RIP3-mediated necrosis of FADD-deficient IECs triggered intestinal inflammation and Paneth cell loss in FADD^{IEC}-KO mice. Moreover, these experiments revealed important roles for TNF, the intestinal microbiota and MyD88-dependent signalling in the pathogenesis of intestinal inflammation in FADD^{IEC}-KO mice. Therefore, regulation of RIP3-mediated programmed necrosis of IECs is essential for the maintenance of intestinal immune homeostasis and the prevention of chronic intestinal inflammation. These studies revealed a novel mechanism regulating the cross talk of epithelial cells with the microbiota and the mucosal immune system and suggest that deregulation of epithelial cell responses

might be an important factor contributing to the pathogenesis of intestinal inflammation. The aforementioned work of the **Pasparakis** team was recently published in *Nature*[28] with the acknowledgment of INFLA-CARE support.

The 'non-canonical' NF-κB2 pathway is considered as a positive regulator of carcinogenesis and is activated in a number of solid and hemopoietic malignancies (reviewed in *Nature Reviews in Cancer* by Neil Perkins[29], P15, with the acknowledgment of INFLA-CARE support). Surprisingly, however, the team of **Jorge Caamano (P12)** found that $Nfkb2^{-/-}$ mice are protected against DSS-induced colonic inflammation (Fig. 1 and data not shown) whereas the related non-canonical NF-kB member RelB responded similar to WT animals. The team tested the role of NF-kB2 in an innate model of colitis. $Nfkb2^{-/-}$ mice were crossed onto a Rag1-deficient background and the double KO mice were put through the DSS colitis protocol. Interestingly, the *Nfkb2/Rag* double KO mice were as susceptible to develop colitis as their Rag KO counterparts. These results indicated that Nfkb2-deficient lymphocytes might have a protective role in this model of colitis. Absence of B and T cells renders the $Nfkb2^{-/-}$ mice as susceptible to DSS-induced colonic inflammation as their $Nfkb2^{+/+}$ littermates.
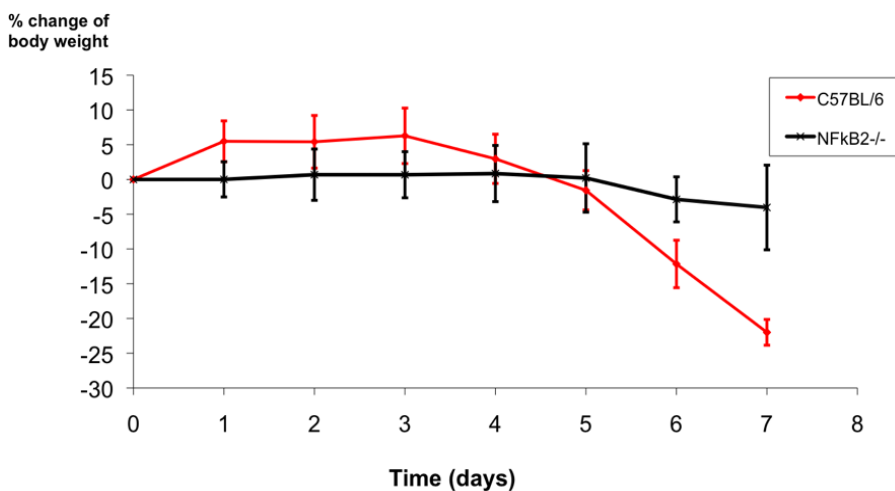
Fig. 11. $Nfkb2^{-/-}$ mice appear to be resistant to acute chemically-induced colitis. The graph shows the percentage change of body weight of $Nfkb2^{-/-}$ (black line) and wild type littermates (C57BL/6) (red line) after treatment with 2% DSS in drinking water. Note the loss of body weight of the WT animals starting at day 5 of treatment compared to the mutant.

To further understand the role of NF-kB2 during colon inflammation P12 characterized the cellular composition of mesenteric lymph nodes from $Nfkb2^{-/-}$ mice and their WT littermates in basal conditions and after DSS-induced colitis. Interestingly, after DSS treatment the cellularity of mLNs from $Nfkb2^{-/-}$ was significantly decreased compared to their WT littermates. In particular the absolute number of CD4+ T cells showed a statistically significant reduction in these organs and in resting conditions the mesenteric lymph nodes of $Nfkb2^{-/-}$ mice presented with increased numbers of TNFα[+] and IFNγ[+] CD4+ T cells as well as Foxp3^{+} regulatory T cells compared to their littermates. After DSS-induced colitis the numbers of these cells decreased to similar levels than in control mice.

Based on our results above, they postulated that if NF-κB2 is important in colon inflammation, mice over-expressing this protein might be prone to develop IBD spontaneously. To test this hypothesis the Caamano team analyzed the phenotype of p100Δ[ki/ki] mice that carry a deletion in the carboxi-terminal region of the *Nfkb2* gene that results in a constitutively activated non-canonical NF-kB pathway. These mice presented with severe colon inflammation at three weeks of age. Detailed

analysis showed that the p100Δ$^{ki/ki}$ mice have a significant increase in the number of isolated lymphoid follicles (ILF) in the colon. Further studies showed that the increased formation of ILFs and colon inflammation in p100Δ$^{ki/ki}$ mice is due to a defect in CD45+ cells. We are currently investigating the impact of nfkb2 deficiency in CAC using the AOM/DSS model and preliminary results suggest that contrary to the protective effect of NF-κB2 on acute colitis described above, these animals develop more and larger tumors than the WT littermates. The mechanism underlining the increased formation of ILFs in p100Δ$^{ki/ki}$ mice and the differential acute vs chronic response of the *Nfkb2$^{-/-}$* mice to the DSS/AOM protocol are being investigated as a collaborative effort between the teams of **Caamano and Eliopoulos**. It is anticipated that a manuscript *will be submitted* for publication in 2014.

P12 performed under WP4, additional studies to test the hypothesis that stromal cells have an essential role in generating special microenvironments for bone marrow derived cells during inflammatory processes. More specifically **P12 (Caamano)** postulated that adipocyte progenitor cells would be induced to become lymphoid tissue stromal cells during inflammatory processes as a response to environmental signals and thus give origin to lymphoid tissues. The results showed that these cells contribute to the development of lymph nodes during mouse development and might also be involved in the formation of ectopic lymphoid organs that perpetuate inflammation during chronic inflammatory diseases as well as contribute to the low level of chronic inflammation seen in obese individuals. This work was recently published in *Immunity* [30] with the acknowledgment of INFLA-CARE support.

The team of **Neil Perkins (P15)** has previously identified crosstalk between the non-canonical NF-κB2 pathway subunit p52 and p53, involving p53 modulation of p52 homodimer transcriptional activity, by inducing a change from p52/Bcl3 to p52/HDAC complexes, in addition to direct recruitment of p52 to p53 target gene promoters.

In the context of INFLA-CARE, P15 has defined a network linking the non-canonical NF-κB pathway to p53 function and the regulation of cell senescence. They have found that in primary human dermal fibroblasts and CD40L stimulated cells from chronic lymphocytic leukemia (CLL) patients, the p52 and RelB NF-κB subunits regulate the expression of EZH2, a component of Polycomb-repressive complex 2 (PRC2
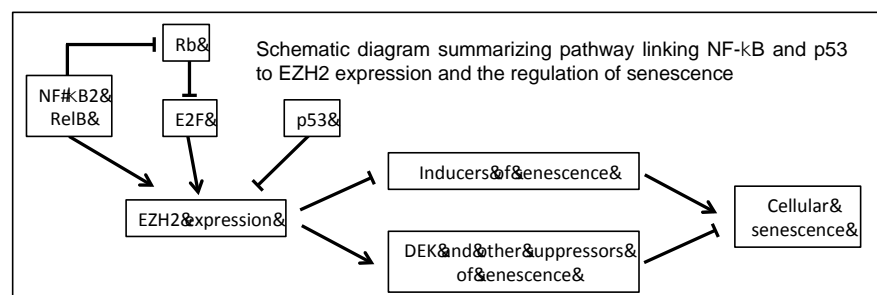
Fig. 12). Further studies showed that EZH2 is the major effector of crosstalk between the alternative NF-κB and p53 pathways and acts to suppress cellular senescence. Analysis of these genes identified the proto-oncogene and histone chaperone DEK as a key component the NF-κB2/EZH2 mediated inhibition of senescence. These results detail a pathway of NF-κB2/p53 crosstalk with the potential to function as mechanism of microenvironment mediated tumour suppressor inhibition in CLL and other malignancies. This work has been *submitted for publication*.

Of further interest, is a proteomic analysis performed by the Perkins' team to identify novel regulators of the p52 NF-κB subunit before and after DNA damage. A number of novel interacting proteins have been identified and follow up experiments are being performed to investigate their function. Among these they identified RPL11, a protein shown to have an important role in activation of p53 in response. This provides a potentially novel mechanism linking NF-κB and p53 and this will be the subject of further investigations currently explored in a collaboration between the laboratory of **Neil Perkins (P15) and Sinisa Volarevic (P16).** Moreover, in the context of INFLA-CARE, The team of Neil Perkins performed a kinome RNAi screen to identify novel regulators of NF-κB/p53 crosstalk. The initial screen has been performed and they have identified kinases and phosphatases that regulate NF-κB transcriptional activity after induction with etoposide, a cancer chemotherapeutic drug and DNA damaging agent that also induces p53. These will be analysed for effects on p53 activity and those shown to have dual NF-κB/p53 activity will be investigated further.

The laboratory of **George Kollias (P4)** in WP5 aimed to elucidate the tissue specific role of two important MAP kinases, Tpl2 and TAK1, in inflammation driven colorectal tumorigenesis. Villin-Cre and ColVI-Cre TAK1 conditional knockout mice exhibited early postnatal lethality. Therefore they focused their studies on Tpl2 and found that the complete Tpl2 knockout (Tpl2$^{D/D}$) mouse strain exhibited increased tumor multiplicity and size at the end of the AOM/DSS treatment, which correlated well with decreased apoptosis, increased proliferation and an altered gene expression profile early during disease progression, revealing thus a tumor suppressive role for Tpl2 in the gut. In order to further elucidate the cellular and molecular mechanisms of Tpl2 actions in the intestine they also used cell- and tissue-specific Tpl2 knockout mice in the same experimental setup and found that Tpl2 deletion in intestinal myofibroblasts (IMFs) but not in intestinal epithelial (IECs) or myeloid cells recapitulated the phenotype observed in the complete knockout mice (Figure 13).
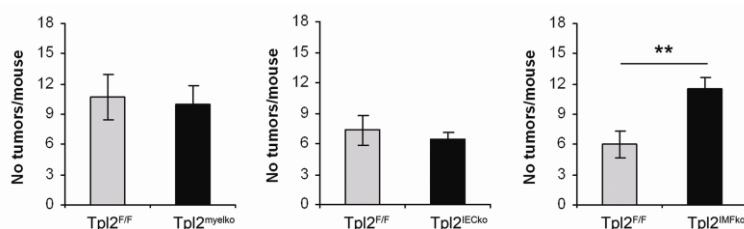
Fig. 13: TPL2 in IMF is required for CAC

Interestingly, both complete (Tpl2$^{D/D}$) and IMF-specific knockout mice (Tpl2^{IMFko}) displayed increased production of HGF in the stroma and activation of the c-Met pathway in adjacent epithelial cells. In vitro experiments confirmed that Tpl2-deficient IMFs upregulated HGF production and became less sensitive to the negative regulation of HGF by TGF-β3. Most importantly, in vivo inhibition of HGF-mediated c-Met activation blocked early, enhanced colon dysplasia in Tpl2-deficient mice, along with increased proliferation and decreased apoptosis, indicating that the HGF/c-Met pathway is the major downstream target of IMF-specific Tpl2 during colitis-associated malignant transformation. These findings establish a mesenchymal specific role for Tpl2 in the regulation of HGF production and suppression of epithelial tumorigenesis and were published in the ***Journal of Clinical Investigation*** (2012)[31] with the acknowledgment of INFLA-CARE support.

Prompted by findings from Text mining bioinformatics tools developed in WP9 and published in ***Bioinformatics*** (2012)[32] with the acknowledgment of INFLA-CARE support, which indicated a

functional link between TPL2 and APC, the team of Ari **Eliopoulos (P1)** was involved in studies showing that the Tpl2 kinase functions to suppress inflammation and cancer in Apc$^{min/+}$ mice. Apc$^{min/+}$/Tpl2$^{-/-}$ mice show exaggerated inflammation and accelerated intestinal carcinogenesis because of reduced IL-10 levels and regulatory T cell (Treg) numbers in the intestines of Tpl2$^{-/-}$ mice. This work which was published in the ***Proceedings of the National Academy of Sciences USA***[33] further highlights the major role of TPL2 in intestinal disease pathogenesis through its action on multiple cellular targets.

Another important MAPK signaling member is p38. **Angel Nebreda's team (P24)** has used genetically modified mice to investigate how p38α downregulation in specific cell types affects inflammation-driven colon cancer. They have found that p38α in myeloid cells does not seem to play a significant role in colon tumorigenesis induced by the AOM/DSS protocol. In contrast, mice lacking p38α in IECs developed more colon tumors than WT mice, but the size of the tumors was about the same. Histological analysis revealed that proliferation indexes were very similar in WT and p38α^{ΔIEC} mice, suggesting that p38α might affect tumor initiation in this model.

Inflammatory cytokines are important for tissue repair but when tissue injury cannot be resolved due to repetitive damage, the resulting compensatory proliferation can promote tumorigenesis. To test this possibility, the Nebreda team treated mice with three cycles of DSS to induce repeated injury/inflammation, but without AOM. As expected, WT mice developed no tumors in the absence of AOM. However, about 60% of the p38αΔIEC mice developed at least 1 macroscopic tumor. Histological examination confirmed the presence of more dysplastic and hyperplastic crypts in p38α^{ΔIEC} mice. Thus, in the absence of p38α, repeated epithelial damage and inflammation induced by DSS results in uncontrolled hyperproliferation of IECs and a pro-tumorigenic environment that ultimately induces colon hyperplasia and tumor formation.

The team also found that *in vivo* intestinal permeability was increased in p38α^{ΔIEC} mice compared to WT mice. Interestingly, epithelial tight junctions in colons from p38α^{ΔIEC} mice were morphologically disrupted and had fewer "kissing" points compared to tight junctions in colon from WT mice. Moreover, the expression of ZO-1, a key component of tight junctions was reduced in p38α^{ΔIEC} mice. Taken together, the altered intestinal homeostasis and impaired epithelial barrier function observed in p38α^{ΔIEC} mice probably account for their enhanced susceptibility to DSS-induced colitis and epithelial damage as well as to colitis-associated colon tumor formation.

Surprisingly, however, whereas p38α functions as suppressor of tumor initiation in the colon, once tumors are formed, p38α was found to promote proliferation and inhibit apoptosis in transformed epithelial cells facilitating colon tumorigenesis. This work which was performed in collaboration with histopathologist **V. Gorgoulis (P11)** has led to the ***submission of 2 papers.***

Identification of genetic determinants of mouse susceptibility to colitis and colon cancer and their relationship to human disease.

One of the objectives of the research performed within this grant was to identify and fine-map genetic factors that determine susceptibility to chronic intestinal inflammation and associated cancer and analyse their immunological role.

The team of **Fiona Powrie (P6)** has identified and fine-mapped a genetic interval on the telomeric part of mouse chromosome 3, which determines susceptibility to *H. hepaticus (Hh)* induced chronic colitis and splenomegaly but not typhlitis. Using a congenic approach and typing of microsatellites and SNPs on multiple recombinant strains they could narrow down this interval to a 1.7Mb region of C57BL/6 RAG-/- (B6) mice which confers protection to the originally susceptible mouse strain 129SvEv.RAG-/- (129). The identified region, designated *Hiccs* (for: **H**elicobacter *hepaticus*-**i**nduced **c**olitis and associated **c**ancer **s**usceptibility) harbours 8 genes, of which 4 are already identified genes (*Neurogenin 2, Tifa, Larp7, Alpk1*), three genes identified by mRNA only (*4930422G04Rik* [abbreviated *493Rik*], *Ap1ar*, and *5730508B09Rik* [*573Rik*]), one hypothetical protein (*LOC100043382*) and 5 micro RNAs (*Mir302a-d* and *Mir367*).

To further analyse the contribution of the *Hiccs* genes to colitis susceptibility they took a systematic approach where they sequenced the congenic 129S7 interval of this region and analysed the gene expression profile in the colon. They determined the sequences of 4 BAC clones of 129 mouse strain origin covering the gene cluster using Illumina Solexa sequencing and the assembled sequences were compared to the published B6 sequence. Two genes, *Alpk1* with 17 and *493Rik* with 25 non-synonymous changes came up as potentially interesting candidate genes. Alpk1 is an alpha protein kinase which was recently identified as a gout susceptibility gene, two of the 17 SNPs identified are actually located in its kinase domain. Expression analysis of colon homogenates by quantitative PCR indicated a significantly higher expression of *Alpk1* in susceptible (recombinant R9) versus resistant (recombinant R17) before and after *Hh* infection.

Bone-marrow transfer experiments using the R17 recombinant revealed that the *Hiccs* locus is acting in hematopoietic cells. Therefore we analysed the expression levels of the candidate genes in sorted CD45+ lamina propria leukocytes (LPL) and in bone-marrow derived macrophages. Increased expression of *Alpk1* and *573Rik* was observed in intestinal leukocytes compared with non-hematopoietic cells and expression of both genes was also induced in bone-marrow derived macrophages upon in vitro stimulation with LPS or *Hh*.

Using the *H*h+AOM model they have found that recombinants carrying the protective *Hiccs* interval have decreased tumour multiplicity suggesting a protective effect of this region for inflammation driven colon cancer. Invading colon carcinoma were found in 75% (12/16) of *Hh*+AOM treated 129.RAG-/- mice with an average of 1.2 lesions per mouse (range 0-4). In contrast the C3B group, which includes mice with the full C3B interval as well as mice with the shorter R17 interval showed a strongly reduced degree of colitis and consequently less mice of this genotype developed colon tumors. Furthermore the average tumor number in C3B.RAG mice was decreased to 0.3 CRC/mouse. Although the incidence rate of colon tumors was reduced to 29% (4/14) of C3B.RAG mice (R17 & C3B mice pooled) the severity observed was not reduced as tumors in C3B mice could in some cases proceed to invading CRC. In summary the *Hiccs* interval, which we found to protect from colitis, leads to a lower incidence rate and frequency of tumors but the severity of tumors is unchanged compared to 129SvEv.RAG. This work has been published in the ***Journal of Experimental Medicine*** [34] with the acknowledgment of INFLA-CARE support.

The role of tumor suppressor p53 and the DDR in CAC
Mutations in p53 have been detected in colitis patients prior to the development of cancer but the activation of DDR during disease progression is not known.

A cohort comprising 160 archival clinical specimens from patients with chronic inflammatory bowel disease (IBD) (Ulcerative colitis, UC, and Crohn's disease, CD); colorectal adenomas (105 specimens) various grades of colorectal carcinomas (over 300 specimens, of which 71 had paired adenoma and carcinoma), and samples from IBD along with the later developed adenoma or carcinoma was evaluated by the team of **Jiri Bartek** (P22) for 15 markers addressing the DNA damage response activation, signaling, checkpoints and repair, and complementary markers of oxidative stress and inflammatory cytokine signaling/NFκB activation. They found that the DDR activation markers are positively expressed in both UC and CD, but not in normal colon. The extent of DDR activation correlates with the overall infiltrate density and DDR signaling is aberrantly activated only in proliferative parts of the IBD crypts (Fig. 14). This may be linked to oxidative damage which although absent in normal colon, it is pronounced in the infiltrating stromal cells of CD and UC lesions, rather in their epithelial cell component. Further studies by the Bartek team in collaboration with SAB member Curt Harris, reported in **PLOS One** [35] a key role for increased macrophage infiltration in UC patients relating to increased cellular senescence and DDR.

The consortium has elucidated the functional crosstalk between DDR and the ARF tumour suppressor, which produced a **Cell Death and Differentiation**[36] and a **Nature Cell Biology** [37] publications.

Oncogenic stimuli trigger the DNA damage response (DDR) and induction of ARF tumor suppressor, both of which can activate the p53 pathway and provide intrinsic barriers to tumor progression. However, the respective timeframes and signal thresholds for ARF induction and DDR activation during tumourigenesis remain elusive. The teams of **Bartek (P22) and Gorgoulis (P11)** have addressed these issues by analyses of mouse models of urinary bladder, colon, pancreatic and skin premalignant and malignant lesions. Consistently, ARF expression occurred at a later stage of tumour progression than activation of the DDR or p16INK4A, a tumour suppressor gene overlapping with ARF. Analogous results were obtained in several human clinical settings, including early and progressive lesions of the urinary bladder, head and neck, skin and pancreas. Mechanistic analyses of epithelial and fibroblast cell models exposed to various oncogenes showed that the delayed up-regulation of ARF reflected a requirement for a higher, transcriptionally-based threshold of oncogenic stress, elicited by at least two oncogenic "hits", compared to lower activation threshold for DDR. Based on these findings, they propose that relative to DDR activation, ARF provides a complementary and delayed barrier to tumour development, responding to more robust stimuli of escalating oncogenic overload.

While commonly regarded as operating independently of each other, some studies proposed that ARF is positively regulated by the DDR. Contrary to either scenario, P22 and P11 found that in human oncogene-transformed cancer cells ATM suppressed, in a transcription-independent manner, ARF protein levels and activity. Mechanistically, ATM activated protein phosphatase 1 (PP1) which antagonized Nek2-dependent phosphorylation of nucleophosmin (NPM), thereby liberating ARF from NPM and rendering it susceptible to degradation by the ULF E3-ubiquitin ligase. In human clinical samples, loss of ATM expression correlated with increased ARF levels and in xenograft and tissue culture models, inhibition of ATM stimulated the tumour-suppressive effects of ARF. These results provide insights into the functional interplay between the DDR and ARF anti-cancer barriers, with implications for tumorigenesis and treatment of advanced tumours. Figure 13 shows a brief

graphical summary of the functional crosstalk between the ATM kinase (DDR signaling) and the turnover of the ARF suppressor protein.

Figure 15. Schematic model of the functional interplay between the ATM kinase - mediated phosphorrylation of PP1 and the protein degradation mechanism of ARF, through regulation of ARF-nucleophosmin (NPM) interaction and ubiquitylation of ARF by the ULF E3 ubiquitin ligase, that targets ARF for accelerated proteasome-mediated degradation in the case of active ATM signaling.

Participant 18 (**Varda Rotter and Moshe Oren**) focused on elucidation of the crosstalk between p53 and NF-κB and its impact on inflammation-related cancer, with particular emphasis on the role of cancer-associated mutant p53 (mutp53) isoforms. These objectives were pursued through a combination of molecular biology approaches employing tissue culture models, and in vivo approaches based on genetically modified mice.

Their results reveal that mutp53 interferes with IκB negative regulation on NF-κB, augments the recruitment of NF-κB to its cognate binding sites within the chromatin allowing for prolonged NF-κB activity and higher cytokine expression, leading to prolongation of the inflammatory response. Participant 18 (Varda Rotter and Moshe Oren) focused on elucidation of the crosstalk between p53 and NF-κB and its impact on inflammation-related cancer, with particular emphasis on the role of cancer-associated mutant p53 (mutp53) isoforms. These objectives were pursued through a combination of molecular biology approaches employing tissue culture models, and in vivo approaches based on genetically modified mice. Their results reveal that mutp53 interferes with IκB negative regulation on NF-κB, augments the recruitment of NF-κB to its cognate binding sites within the chromatin allowing for prolonged NF-κB activity and higher cytokine expression, leading to prolongation of the inflammatory response.

To address the impact of p53 mutations on CAC, Oren-Rotter teams also employed mutp53 "knock-in" mice harbouring a mutp53 allele; this mutation, p53Arg172His, is the mouse equivalent of the human hot-spot mutation p53R175H, which is frequently observed in many types of human cancer including colorectal cancer (CRC).

DSS was employed in either a chronic (three intermittent cycles) or acute (single cycle) setting. They found that both genotypes were similarly affected by the first DSS cycle, exhibiting comparable weight loss (Fig. 1a). However, as mice progressed through the chronic DSS protocol, a marked difference became apparent: whereas +/- mice seemed to recover quite well from repeated DSS cycles, +/m mice displayed progressive weight loss, indicating failure to recover and sustained tissue

damage (Fig. 16). Histological examination revealed chronic persistent inflammation and injury in the colons of +/m mice, including loss of glands, glandular architectural distortion, persistent ulcers, extensive edema and inflammatory infiltrates, whereas +/- colons appeared to be undergoing effective tissue repair (Fig. 16). Thus, the presence of a single mutp53 allele suffices to hamper tissue recovery and renders the mice markedly more susceptible to the deleterious effects of DSS.
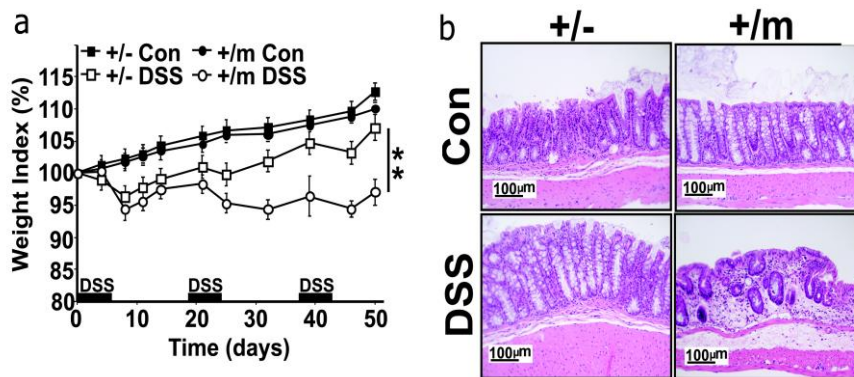
Fig. 16. Mice expressing mutp53 are excessively susceptible to DSS. a.p53+/- and p53+/m mice were either treated with 2% DSS at the indicated time windows or left untreated (Con). Body weight was monitored throughout the indicated period. Values represent average relative weight normalized to the weight at the start of the treatment; bars indicate standard errors. **=p-value< 0.01. b. Colons of p53+/- and p53+/m mice, either untreated (Con) or treated with DSS, were collected at day 60 and subjected to histopathological analysis. Arrowheads denote areas of gland loss; asterisk denotes edema.

It is of particular note that unlike in sporadic human CRC, where p53 mutations are a late event, such mutation are believed to be a very early, if not the earliest, driver event in human CAC. On the basis of these facts and findings, we therefore conclude that the model developed within the context of the INFLA-CARE project, where p53 mutations are present a priori and which mimics closely the known histopathological features of human colitis-associated CRC, is a very faithful representation of the human disease and can thus serve for a variety of pertinent preclinical studies. The work of Rotter and Oren which has been summarized above has led to 2 publications in *Cancer Cell*[38], and *Journal of Cell Science*[39] with the acknowledgment of INFLA-CARE support.

Post-transcriptional regulation of CAC

The ERK and p38 MAPKs influence gene expression at multiple levels, including post-transcriptional regulation through RBPs. To decipher the involvement of the RBP HuR in inflammation and intestinal cancer, the team of **Dimitris Kontoyiannis (P4)** generated mice lacking HuR in myeloid-lineage cells and revealed their enhanced sensitivity to CAC. This phenotype was governed by high levels of pro-inflammatory cytokines due to lack of inhibitory effects on the nducible translation and/or stability of their mRNAs. Conversely, myeloid overexpression of HuR induced posttranscriptional silencing, reduced inflammatory profiles, and protected mice from colitis and cancer (Figure 17). This analysis revealed that HuR may be a key controller of RBP-complexes governing macrophage activation, polarization and migration which if failed it can contribute to tumorigenesis; it also demonstrated the potential of harnessing the effects of HuR for clinical benefit against pathologic inflammation and cancer. This work has been published in *Journal of Clinical Investigation* (2012)[40].

The functions of HuR in intestinal epithelia are however more complex than in immune cells; mice engineered to lack or overexpress HuR in intestinal epithelia, provides evidence for differential functions in selective steps of the epithelial transformation process. Under conditions that favor the development of colitis associated cancer (CAC), the loss of epithelial HuR exacerbated the severity of inflammatory degeneration in the affected colons but suppressed the tumorigenic process due to a significant increase in epithelial death; conversely the enhanced expression of HuR in the same locality, led to a profound increase in epithelial survival and tumor burden. However, the pro-tumorigenic activities of HuR in CAC contrasted its functions in modeled familial adenomatus polyposis, driven by oncogenic *Apc* mutations. In that setting, the loss of epithelial HuR augmented the process of neoplastic transformation, tumor burden and mortality due to changes in the proliferative status of epithelial progenitors. Using an *in vivo* tagging system to trace the HuR:RNA and protein interactome in developing tumors, we identified key networks associating with HuR for the regulation of RNAs encoding selective inflammatory stress responders and epigenetic modifiers relating to epithelial renewal. Collectively, our data highlight HuR's function as a balancing node between epithelial degeneration and regeneration thus acting to safeguard against the totality of the tumorigenic process (*Christodoulou-Vafeiadou et al.*, **in preparation**).
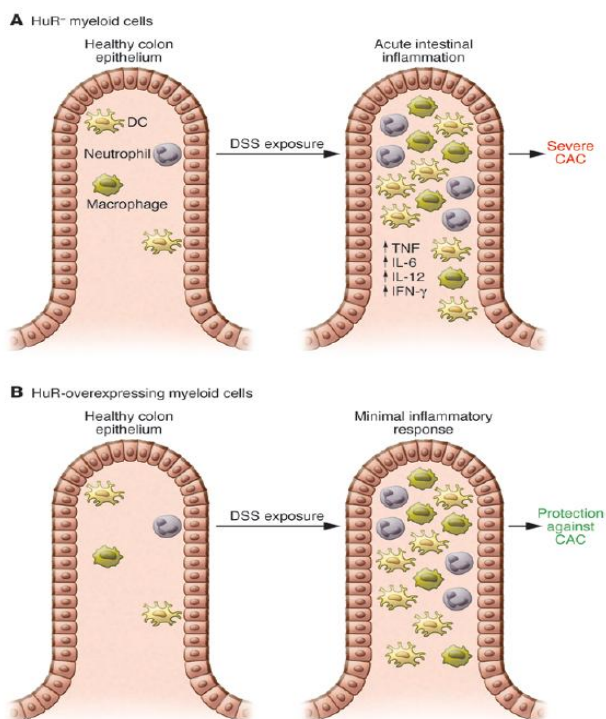
Figure 17. Summary of biomedical findings on myeloid HuR by Yiakouvaki et al., as commented by Shultz & Chalfant, 2012, J. Clin. Invest, 122:16-19. (A) Following exposure to DSS, mice devoid of HuR in the myeloid lineage(MKO mice) exhibited faster colitis onset and higher disease activity in comparison to control mice. Cultures and RNA extracts from the colons of MKO mice treated with DSS revealed increased expression of the proinflammatory cytokines TNF, IL-6, IL-12, and INF-γ as compared with control mice treated with DSS. MKO mice were also sensitized to CAC and developed higher numbers of tumors as well as increased tumor size and grading as compared with control mice. (B) Reversely, the study by Yiakouvaki and colleagues indicates that mice overexpressing myeloid HuR exhibit delayed onset and severity of colitis and resistance to the development of CAC.

To further explore how environmental stress factors could control cancer, especially via epigenetic modifications, the **Penninger team** (P2) homed onto a protein termed Epc1. Epc1 has been implicated in epigenetic regulation by the polycomb complex. They identified Epc1 as a major modifier of Ras-induced hyperplasia using a – yet unpublished – Drosophila melanogaster model for Ras-driven oncogenesis. The role of Epc1 is being further addressed in other oncogene models in the fly. We also generated an Epc1 floxed allele to delete Epc1 in defined tissues. Preliminary results indicate that tissue specific deletion of Epc1 results in markedly enhanced DSS/AOM induced colon cancer, increased sex hormone-driven breast cancer (MPA/DMBA model) as well as markedly

enhanced KRas-driven lung cancer. Thus, they have uncovered a novel epigenetic regulator that, based on current preliminary data, is a key pathway to couple environmental stress to tumorigenesis.

Other malignancies

Manuela Baccarini (P17) has also investigated undwer WP5 the mechanism by which the ablation of both BRaf and CRaf in the epidermis gives rise to both local (in the dermis) and systemic inflammation and ultimately to a progressive disease strongly resembling human atopic dermatitis (Fig. 18). We could show that compound Raf ablation causes a delay in the development of the epidermis and its appendages, particularly the hair; as a result of this delay, the expression of E-cadherin and claudin in the epidermis of neonates is strongly reduced, resulting in the weakening of tight junctions and in the disruption of the inside-out barrier function. In addition, Raf-deficient keratinocytes hyperreact to inflammatory stimuli commonly present in the skin, such as TNF-α or interleukin-β. This results in the hyperproduction of cytokines, which correlates with increased JNK and p38 activation and can be reverted by JNK inhibitors. Thus, ablation of both B-Raf and C-Raf results in the deregulation of inflammatory MAP-Kinases (Raguz et al., **manuscript in preparation**).
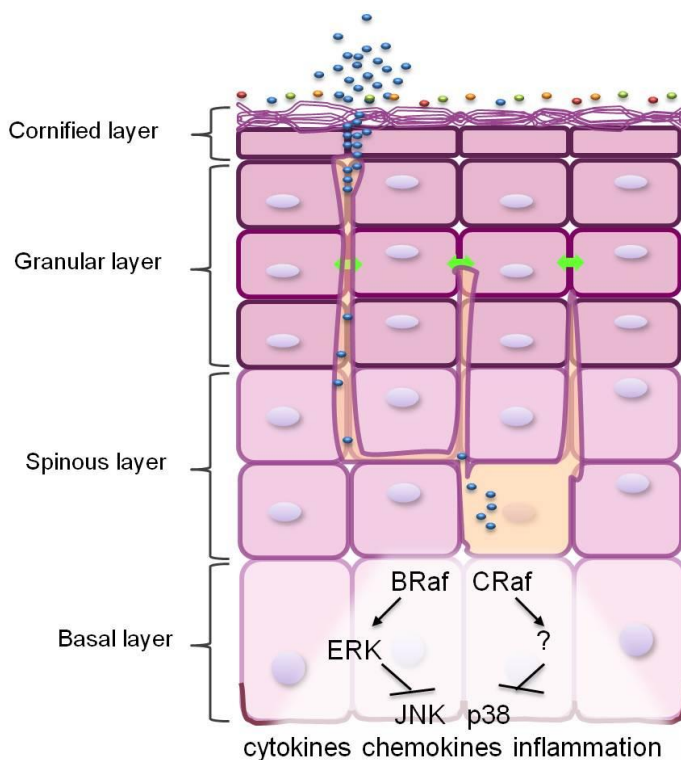
Figure 18 – Compound ablation of B+Craf in the mouse epidermis induces a phenotype akin to human atopic dermatitis. B+CRaf ablation results in a delay in the maturation of the epidermal barrier, particularly of tight junction formation (green double arrows). As a result of this, dendritic cells in the epidermis are exposed to antigens from the environment. In addition, in the absence of B+CRaf, the inflammatory MAPKs JNK and p38 are activated in ERK-dependent and – independent manners, resulting in the production of cytokines and chemokines and in the onset of both local and systemic inflammation.

The team of **Jiri Bartek** (P22) reports in ***Cancer Cell*** (2013)[41] the generation and characterization of a mouse model of myelogenous leukemia induction by a knocked-in regulatable MLL-ENL fusion oncogene mimicking human disease and documented the role of ATR and ATM signaling as an anti-leukemic barrier in vivo, as well as a rich spectrum of inflammatory cytokines (including TNF) and their links with DDR. Thus, activation of MLL-ENL led to hyperproliferation in both bone marrow and spleen, and this was accompanied by markers of replication stress. At around 7-8 months of continuous oncogene activation, senescence phenotype replaced the hyperproliferation, and this was

particularly robust in the bone marrow, but also evident in the spleen; only with a much longer latency, about half of the animals developed myeloid leukemia including large numbers of leukemia stem cells. The obvious tissue differences were apparently attributable to the fact that unlike in spleen where only ATR-Chk1 signaling was predominant, the pronounced senescence in the bone marrow was accompanied by parallel activation of the ATM-Chk2-p53 pathway. Interestingly, a large spectrum of cytokines and cytokine receptor genes were found activated at the transition period and during senescence. Collectively, these results provided novel and important insights into the functional interplay between oncogene-induced replication stress, DNA damage signaling and inflammatory cytokine signaling (including TNF) network in an in vivo model of tumorigenesis, closely mimicking human disease.

The complex relationship between cancer and the immune system is further highlighted by a seminal paper published in *Science*[2] by a team of researchers that includes INFLA-CARE participants **Laurence Zitvogel (P5)** as co-senior author and **Josef Penninger (P2)** as co-author showing that chromosomal content in a tumor is controlled indirectly by an immunosurveillance mechanism which ensures the elimination of hyperploid cells. This work which acknowledges INFLA-CARE support, demonstrated that hyperploid cells become immunogenic because of a constitutive endoplasmic reticulum stress response resulting in the aberrant cell surface exposure of calreticulin. CRT facilitates the phagocytosis of stressed and dying cells by macrophages as well as by antigen-presenting dendritic cells and it thus part of a barrier mechanism to restrain tumor growth [2].

Exploiting INFLA-CARE research towards novel therapeutic or prognostic tools

Hepatocellular carcinoma.

<u>**5-HT$_2$B antagonists**</u>: In a paper recently published in *Nature Medicine* by **Derek Mann's group** (P15, acknowledging INFLA-CARE support)[42], a paracrine signaling pathway in which fibrogenic hepatic stellate cells (HSCs) repress hepatocyte proliferation was uncovered. Serotonin (5-HT) is important for stimulating hepatocyte proliferation via 5-HT$_2$A receptors expressed on hepatocytes. However, serotonin also stimulates the expression of anti-proliferative and pro-fibrogenic TGFβ1 by HSCs. This latter effect is controlled by 5-HT$_2$B receptors which are expressed on HSCs. Hence, in the disease liver, the pro-proliferative influence of serotonin via hepatocyte 5-HT$_2$A receptors is opposed by the anti-proliferative influence of serotonin via 5-HT$_2$B/TGFβ1 signaling from HSC. P15 found that antagonists of 5-HT$_2$B signaling block TGFβ expression by HSC, stimulate hepatocyte proliferation and suppress liver fibrosis. As it is possible to synthesise highly selective 5-HT$_2$B antagonists and as this class of compounds are proven to be safe for use in man, the implications of this work are that 5-HT$_2$B antagonists should now be tested as preventative medicines in chronic liver disease. Indeed, this work has interested a number of pharmaceutical partners who are considering the 5-HT2B/ERK pathway for therapeutic development.

<u>**C1-3 liposome vehicle for the delivery of siRNAs**</u>: The team of **Derek Mann (P15)** has collaborated with the **Ponzoni group (P5)** to utilize the myofibroblast specific scAb C1-3 antibody and develop a <u>C1-3-Liposome delivery vehicle</u> that provides selective targeting of molecules such as drugs and siRNA to liver myofibroblasts. They have established a reproducible and robust

methodology for producing the C1-3 coated liposomes and have shown that *in vivo* delivery of the drug doxorubicin when packaged into the C1-3 liposomes will selectively deplete alpha-smooth muscle actin positive myofibroblasts. They are currently combining this technology with NF-κB inhibitors to promote the apoptosis of myofibroblasts, resolution of fibrosis and prevention of HCC in mouse models including the nfkb1-/- and CCL4/DEN models described above.

Colitis-associated colon cancer (CAC)

TNF: The experience from the AOM-DSS CAC platform has been used to extend the application of this protocol to humanized mice available in Biomedcode (P20), such as the Tg1278/TNFKO (normally regulated human TNF) and hTNFR1KI/KI (the endogenous mouse TNFR1 replaced by its human homologue) mice that can be used for drug efficacy studies of therapeutics developed by other InflaCare partners. Moreover, in the frame of further standardization of the preclinical drug evaluation platform based on the hTNFR1KI/KI mice P20 have evaluated the therapeutic potential of an anti-hTNFR1 therapeutic (previously positively evaluated by us in arthritis and multiple sclerosis models). This study was based on a recent publication[43] showing that anti-TNF reduces tumorigenesis and that the effect depends on TNFR1. One first round of experiments had indicated some therapeutic potential for the anti-hTNFR1 treatment, though without statistical significance (Figure 19). Nevertheless repeated efforts by Biomedcode using modified protocols and a new preparation of the anti-hTNFR1 did not lead to a statistically significant improvement of the pathology.
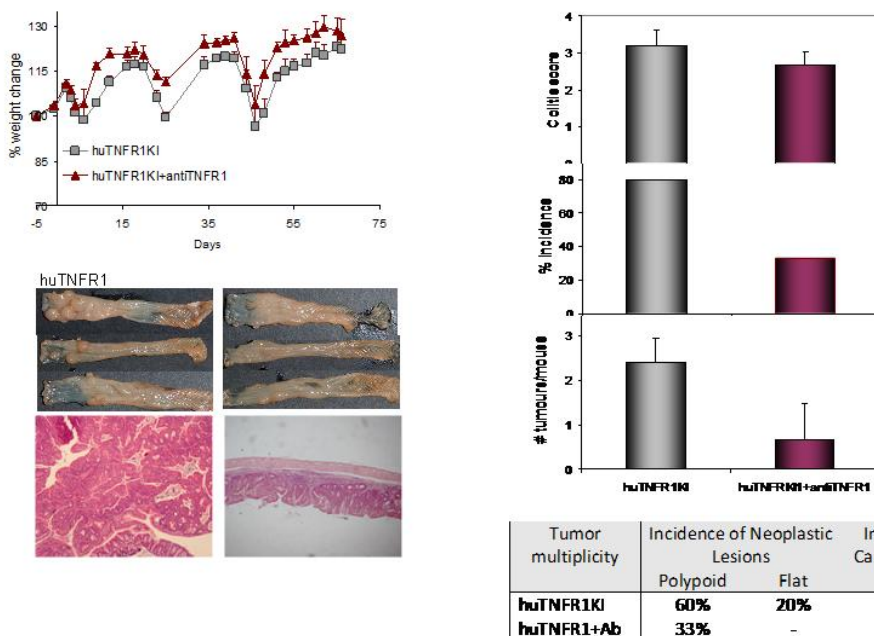
Figure 19: Evaluation of anti-hTNFR1 in hTNFR1KI-based CAC preclinical evaluation platform. Study performed in year 2 of the project giving promising results on the potential role of antihTNFR1 as a CAC therapeutic.

Tumor multiplicity	Incidence of Neoplastic Lesions		Invasive Carcinoma
	Polypoid	Flat	
huTNFR1KI	60%	20%	20%
huTNFR1+Ab	33%	-	-

However, the team of Biomedcode in collaboration with the group of George Kollias have uncoupled the anticancer and proinflammatory effects of TNF and developed a strategy for therapeutic application of TNF. Whilst TNF was first described as a potent anti-tumor agent, the severe side-effects associated with administration of high doses of this cytokine prohibit its widespread use as

therapy. In a paper which was recently published in the ***Journal of Clinical Investigation***[44] with the acknowledgment of INFLA-CARE support, they report that deletion of one functional *Tnfrsf1a* allele leads to complete protection against challenge with a dose of TNF that is at least 40 times of LD_{100} without affecting physiological functions of TNF, such as antibacterial resistance and secondary lymphoid organ structure and function. Importantly, both in normal mice (with anti-mouse p55TNFR antibodies) and in newly generated mutant mice humanized for the *TNFRSF1A* gene systemic inhibition of p55TNFR using antibodies was clearly protective against the unwanted TNF effects, while the antitumor activity remained functional and led to an increase of 4 to 5 times of LD_{50}.

In conclusion, this work has identified a way to increase the safety of the anticancer effect of TNF, by partly neutralizing p55TNFR allowing the safe administration of higher, tumor-destructive doses of TNF. This approach may be directly applicable to the clinic.

IL-1α neutralization: The team of Ron Apte (P3) has performed neutralisation of IL-1α which led to a decrease in both clinical and colon manifestations of CAC, based on the analysis of the relative roles of IL-1α and IL-1β. There were no significant changes in clinical or histological scores in mice treated with anti-IL-1β. The effect of IL-1Ra was also less pronounced than those found with treatment with anti- IL-1α. This analysis is now pursued using the respective conditional knock-out mice. P3 has also established a syngeneic model of orthotopic colorectal cancer and is currently assessing the effects of IL-1a neutralization on tumor growth using a combination of MRI and in vivo measurements. The results are expected to become available within the next 3 months.

Gut microbiota, anticancer immunity and therapy. Some anti-neoplastic agents, including cyclophosphamide (CTX), reinstate failed immunosurveillance as a mechanism of their therapeutic anticancer effects. The Zitvogel team (P7) has found that CTX induces profound changes in the small intestinal microbiota and provokes the translocation of selected species of Gram$^+$ bacteria into secondary lymphoid organs, hence stimulating the generation of a specific subset of "pathogenic" T helper 17 (Th17) cells. Germ free mice or hosts treated with antibiotics killing Gram$^+$ bacteria exhibited reduced pTh17 responses and relative chemoresistance to CTX unless adoptively transferred with pTh17 cells. Moreover, creating a dysbiosis (monoassociation with *P. distasonis* or segmented filamentous bacteria) inhibited the activity of anticancer chemotherapeutics. These results unveil the unsuspected role of gut microbiota in shaping anticancer immunosurveillance which may impact on the application of future therapeutic strategies (Viaud et al, ***Science***, under review).

Breast cancer

RANK-L: Recently, an anti-RANKL (receptor activator of NF-κB ligand) antibody, which was developed for the treatment of osteoporosis, was shown to be effective in inhibition of bone metastasis in prostate cancer [45]. Work performed by the **Penninger group** which was published in ***Nature*** with INFLA-CARE support[46], showed that the RANKL/RANK system controls the incidence and onset of progestin-driven breast cancer. Specifically, they showed that the in vivo administration of medroxyprogesterone acetate (MPA), used in millions of women for hormone replacement therapy and contraceptives, triggers massive induction of RANKL in mammary-gland epithelial cells and that genetic inactivation of RANK in mammary-gland epithelial cells prevents the pro-tumorigenic effects of MPA.

Prognostic value of immune component gene polymorphisms: The work of **Laurence Zitvogel (P7)** in the context of WP7 underscores a bidirectional tumor-host interaction during therapeutic intervention[47-49] (reviewed in Ref. [50,51] with acknowledgment of INFLA-CARE support). Failure to activate an immune response compromises the success of anticancer chemotherapies and can be predicted by specific molecular signatures. Predictors in BC include i) <u>at the level of the tumor</u> which is expected to emit « danger signals » (such as markers of ER stress response and HMGB1 release and autophagy) and ii) <u>at the level of the host</u> that sense such danger signals (such as SNPs in TLR4/Myd88, P2RX7 genes and others) that will both <u>predict the probability of a patient to relapse</u> following anthracycline-based therapy. For example, TLR4Asp299Gly loss of function single nucleotide polymorphism predicts a worse response to oxaliplatine in metastatic colon cancer individuals compared with the normal allele of TLR4 and a shorter time to progression in breast cancer females treated in adjuvant with anthracyclines (published in ***Nature Medicine***[48] and ***Oncogene***[52] with the acknowledgment of INFLA-CARE support).

Based on results obtained in several retrospective analyses of BC specimen, P5 have designed and patented a kit of immunohistochemistry composed of five antibodies recognizing the candidate gene products of the ER stress, autophagy, apoptosis response on the initial BC core biopsy (IHC scoring based on CRT, HMGB1, LC3, ERp57, EIF2ap). Secondly, using a biaised (based on animal studies) and unbiaised (multiplex 384 SNP genotyping based on fluorescent PCR) selection of immune SNPs associated with the absence of pathological complete response or with shorter time to progression in a cohort of >300 N$^+$ breast cancer patients, P5 could design a 3 immune gene-based signature using a multivariate logistic regression analysis that predict clinical outcome, independently of classical clinicopathological factors validated in BC. This predictor that detects functional defects in anticancer immune responses will allow the design of appropriate compensatory therapies targeting these immune defects not only in BC but eventually in other cancers.

Other human malignancies

Prognostic value of NKp30: The team of **Laurence Zitvogel** at IGR **(P7)** reported in ***Nature Medicine***[53] that NK cells are major components of gastrointestinal sarcoma and high grade neuroblastoma endowed with prognostic value in large cohorts of patients. Moreover, the NK specific NKp30 receptor is often downregulated in lesions and subjected to a post-transcriptional regulation whereby NKp30 isoforms dictate the prognosis of patients[53]. In melanoma, the NKG2D receptors prevail to influence long term survival and polymorphisms in MICA/NKG2D ligands are also associated with prognosis (Romero, ***Cancer Res, in revision***).

Liposome vehicle for the delivery of siRNAs: The team of Mirco Ponzoni (P5) has developed liposomal formulations for siRNA delivery and applied it in neuroblastoma cancer models as i*n vivo* proof-of-concept of functionality. They showed that this novel neuroblastoma-targeted lipid nanoparticles strategy for the delivery of anti-*ALK* siRNA is safe and highly effective in mouse models of neuroblastoma, inducing selective block of tumor growth and apoptosis and inhibiting angiogenesis. This work was published in ***Molecular Therapy***[54] with the acknowledgment of INFLA-CARE support. Ongoing studies aim to utilize this approach towards the *in vivo* application of siRNA therapeutics in HCC and CAC in collaboration with the teams of Ari Eliopoulos, Derek Mann and Manolis Pasparakis.

Potential impacts and use of the results.

Chronic inflammation and cancer are critical health issues for Europe and represent a major focus for research funded by the European Commission. Results obtained from INFLA-CARE research will have major scientific impact, providing clues to the dominant mechanisms operating to trigger tumor initiation in a chronic inflammatory state.

A challenge in the field has been the development of pre-clinical models which closely mimic disease progression in humans and could stimulate efforts towards characterisation of novel therapies. A major achievement of the consortium during its operation has been the establishment of state-of-the-art genetic and chemical in vivo models which closely mimic inflammation-associated human malignancies, including colitis-associated colon cancer (CAC), hepatocellular carcinoma (HCC), lung and breast tumorigenesis. Transplantable in vivo models have also been developed to assist the characterization of the impact of immune cells on response to chemotherapy and metastasis. These models are coupled to genetically engineered mice carrying defects in various cytokines and signalling mediators aiming to determine their physiological role in inflammatory conditions and cancer. The technological capability and proven expertise in disease modelling represent a major strength and highly competitive aspect of INFLA-CARE and is continuing to generate major breakthroughs in the field.

Thus, the consortium has already defined unprecedented roles for soluble mediators and signalling molecules in colitis and colitis-associated colon cancer (CAC), characterised cytokines, lymphocytic populations and signalling molecules important for response to therapy and defined the impact of transcriptional and post-transcriptional networks in inflammation and cancer. Exciting new findings have also emerged through the study of DNA damage response (DDR) and DDR-related molecular pathways and the identification of susceptibility loci in CAC. In parallel with mouse disease models, the team has employed histological and functional assays in primary human tumors and cell lines to evaluate the involvement of lymphocytic populations and molecular pathways in human cancer associating with chronic inflammation.

Currently, there is no systematic attempt to target the inflammatory component of tumors as a therapeutic strategy and our studies have enabled breakthroughs in translational research directly amenable for clinical applications. The consortium has identified molecular signatures and genes which predict the probability of a colon or breast cancer patient to relapse following therapy. An immunohistochemistry kit has been designed and patented which, together with 3 immune gene-based signature, may predict clinical outcome to therapy independently of classical clinicopathological factors validated in breast cancer. Agents which neutralize or carefully modulate the expression of certain pro-inflammatory factors have been developed and shown to possess anti-tumor activity in animal models. Novel liposomal vehicles have been developed suitable for the *in vivo* application of siRNA therapeutics in neuroblastoma, HCC and CAC. Work performed by the consortium paves the way for testing of serotonin receptor antagonists as preventative medicines in chronic liver disease and has interested a number of pharmaceutical partners who are considering the this pathway for therapeutic development.

Moreover, by incorporating the accumulating knowledge into systems biology platforms, we have developed new hypotheses which are likely to influence and shape future research in the field of

'inflammation & cancer'. The scientific impact of the INFLA-CARE project is highlighted by a large number of papers (**149 publications**) published during the period 01/01/2009 – 30/08/2013, many of which in high profile scientific journals including *Nature, Nature Genetics, Nature Medicine, Science, Cancer Cell, J. Exp. Med., J. Clin. Invest., Proc. Natl. Acad. Sci. USA, EMBO J, Mol. Cell, Cancer Res., Nat. Rev. Cancer*, etc. and our involvement in more than 200 dissemination activities.

Finally, through a coherent set of measures which include organisation of Summer Schools, participation in conferences and public lectures, publication and circulation of a e-Newsletter, etc. the consortium has worked to disseminate the achievements of its members to the wider scientific community and the lay public.

REFERENCES

1.   Dunn, G.P., Old, L.J. & Schreiber, R.D. The three Es of cancer immunoediting. *Annu Rev Immunol* **22**, 329-60 (2004).
2.   Senovilla, L. et al. An immunosurveillance mechanism controls cancer cell ploidy. *Science* **337**, 1678-84 (2012).
3.   Maloy, K.J. & Powrie, F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* **474**, 298-306 (2011).
4.   Alberg, A.J., Brock, M.V. & Samet, J.M. Epidemiology of lung cancer: looking to the future. *J Clin Oncol* **23**, 3175-85 (2005).
5.   Malkinson, A.M. Role of inflammation in mouse lung tumorigenesis: a review. *Exp Lung Res* **31**, 57-82 (2005).
6.   Smith, C.J., Perfetti, T.A. & King, J.A. Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. *Inhal Toxicol* **18**, 667-77 (2006).
7.   Ehlken, H. et al. Hepatocyte IKK2 protects Mdr2-/- mice from chronic liver failure. *PLoS One* **6**, e25942 (2011).
8.   Perugorria, M.J. et al. Tumor progression locus 2/Cot is required for activation of extracellular regulated kinase in liver injury and toll-like receptor-induced TIMP-1 gene transcription in hepatic stellate cells in mice. *Hepatology* **57**, 1238-49 (2013).
9.   Ehrenreiter, K. et al. Raf-1 addiction in Ras-induced skin carcinogenesis. *Cancer Cell* **16**, 149-60 (2009).
10. Karreth, F.A., Frese, K.K., Denicola, G.M., Baccarini, M. & Tuveson, D.A. C-Raf is required for the initiation of lung cancer by K-Ras. *Cancer Discov* **1**, 128-136 (2011).
11. Blasco, R.B. et al. c-Raf, but Not B-Raf, Is Essential for Development of K-Ras Oncogene-Driven Non-Small Cell Lung Carcinoma. *Cancer Cell* **19**, 652-63 (2011).
12. Naugler, W.E. et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* **317**, 121-4 (2007).
13. Ehrenreiter, K. et al. Raf-1 regulates Rho signaling and cell migration. *J Cell Biol* **168**, 955-64 (2005).
14. Golomb, L. et al. Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis. *Mol Cell* **45**, 222-32 (2012).
15. Bursac, S. et al. Mutual protection of ribosomal proteins L5 and L11 from degradation is essential for p53 activation upon ribosomal biogenesis stress. *Proc Natl Acad Sci U S A* **109**, 20467-72 (2012).
16. Schramek, D. et al. The stress kinase MKK7 couples oncogenic stress to p53 stability and tumor suppression. *Nat Genet* **43**, 212-9 (2011).
17. Oehrl, W., Cotsiki, M. & Panayotou, G. Differential regulation of M3/6 (DUSP8) signaling complexes in response to arsenite-induced oxidative stress. *Cell Signal* **25**, 429-38 (2013).
18. Vougioukalaki, M., Kanellis, D.C., Gkouskou, K. & Eliopoulos, A.G. Tpl2 kinase signal transduction in inflammation and cancer. *Cancer Lett* **304**, 80-9 (2011).
19. Gkirtzimanaki, K. et al. TPL2 kinase is a suppressor of lung carcinogenesis. *Proc Natl Acad Sci U S A* **110**, E1470-9 (2013).

20.   Sgantzis, N., Yiakouvaki, A., Remboutsika, E. & Kontoyiannis, D.L. HuR controls lung branching morphogenesis and mesenchymal FGF networks. *Dev Biol* **354**, 267-79 (2011).

21.   Carmi, Y. et al. Microenvironment-derived IL-1 and IL-17 interact in the control of lung metastasis. *J Immunol* **186**, 3462-71 (2011).

22.   Carmi, Y. et al. The role of IL-1beta in the early tumor cell-induced angiogenic response. *J Immunol* **190**, 3500-9 (2011).

23.   Terme, M. et al. Cancer-induced immunosuppression: IL-18-elicited immunoablative NK cells. *Cancer Res* **72**, 2757-67 (2012).

24.   Terme, M. et al. IL-18 induces PD-1-dependent immunosuppression in cancer. *Cancer Res* **71**, 5393-9 (2012).

25.   Bersudsky, M. et al. Non-redundant properties of IL-1alpha and IL-1beta during acute colon inflammation in mice. *Gut* (2013).

26.   Carmi, Y. et al. The role of IL-1beta in the early tumor cell-induced angiogenic response. *J Immunol* **190**, 3500-9 (2013).

27.   Vlantis, K. et al. Constitutive IKK2 activation in intestinal epithelial cells induces intestinal tumors in mice. *J Clin Invest* **121**, 2781-93 (2011).

28.   Welz, P.S. et al. FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature* **477**, 330-4 (2011).

29.   Perkins, N.D. The diverse and complex roles of NF-kappaB subunits in cancer. *Nat Rev Cancer* **12**, 121-32 (2012).

30.   Benezech, C. et al. Lymphotoxin-beta receptor signaling through NF-kappaB2-RelB pathway reprograms adipocyte precursors as lymph node stromal cells. *Immunity* **37**, 721-34 (2012).

31.   Koliaraki, V., Roulis, M. & Kollias, G. Tpl2 regulates intestinal myofibroblast HGF release to suppress colitis-associated tumorigenesis. *J Clin Invest* **122**, 4231-42 (2012).

32.   Papanikolaou, N. et al. BioTextQuest: a web-based biomedical text mining suite for concept discovery. *Bioinformatics* **27**, 3327-8 (2011).

33.   Serebrennikova, O.B. et al. Tpl2 ablation promotes intestinal inflammation and tumorigenesis in Apcmin mice by inhibiting IL-10 secretion and regulatory T-cell generation. *Proc Natl Acad Sci U S A* **109**, E1082-91 (2012).

34.   Kirchberger, S. et al. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J Exp Med* **210**, 917-31 (2013).

35.   Sohn, J.J. et al. Macrophages, nitric oxide and microRNAs are associated with DNA damage response pathway and senescence in inflammatory bowel disease. *PLoS One* **7**, e44156 (2012).

36.   Evangelou, K. et al. The DNA damage checkpoint precedes activation of ARF in response to escalating oncogenic stress during tumorigenesis. *Cell Death Differ* (2013).

37.   Velimezi, G. et al. Functional interplay between the DNA-damage-response kinase ATM and ARF tumour suppressor protein in human cancer. *Nat Cell Biol* **15**, 967-77 (2013).

38.   Cooks, T. et al. Mutant p53 prolongs NF-kappaB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. *Cancer Cell* **23**, 634-46 (2013).

39.   Solomon, H. et al. Various p53 mutant proteins differently regulate the Ras circuit to induce a cancer-related gene signature. *J Cell Sci* **125**, 3144-52 (2012).

40.   Yiakouvaki, A. et al. Myeloid cell expression of the RNA-binding protein HuR protects mice from pathologic inflammation and colorectal carcinogenesis. *J Clin Invest* **122**, 48-61 (2012).

41.   Takacova, S. et al. DNA damage response and inflammatory signaling limit the MLL-ENL-induced leukemogenesis in vivo. *Cancer Cell* **21**, 517-31 (2013).

42.   Ebrahimkhani, M.R. et al. Stimulating healthy tissue regeneration by targeting the 5-HT(2)B receptor in chronic liver disease. *Nat Med* **17**, 1668-73 (2011).

43.   Popivanova, B.K. et al. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest* **118**, 560-70 (2008).

44.   Van Hauwermeiren, F. et al. Safe TNF-based antitumor therapy following p55TNFR reduction in intestinal epithelium. *J Clin Invest* **123**, 2590-603 (2013).

45.   Hurst, L.C. et al. Injectable collagenase clostridium histolyticum for Dupuytren's contracture. *N Engl J Med* **361**, 968-79 (2009).

46.   Schramek, D. et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. *Nature* **468**, 98-102 (2010).

47.   Zitvogel, L. & Kroemer, G. The dilemma of anticancer therapy: tumor-specific versus immune effects. *Blood* **112**, 4364-5 (2008).

48.   Apetoh, L. et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* **13**, 1050-9 (2007).

49.   Viaud, S. et al. Cyclophosphamide induces differentiation of Th17 cells in cancer patients. *Cancer Res* **71**, 661-5 (2010).

50.   Ma, Y. et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. *Semin Immunol* **22**, 113-24 (2010).

51.   Ma, Y. et al. How to improve the immunogenicity of chemotherapy and radiotherapy. *Cancer Metastasis Rev* **30**, 71-82 (2011).

52.   Tesniere, A. et al. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* **29**, 482-91 (2010).

53.   Delahaye, N.F. et al. Alternatively spliced NKp30 isoforms affect the prognosis of gastrointestinal stromal tumors. *Nat Med* **17**, 700-7 (2011).

54.   Di Paolo, D. et al. Selective therapeutic targeting of the anaplastic lymphoma kinase with liposomal siRNA induces apoptosis and inhibits angiogenesis in neuroblastoma. *Mol Ther* **19**, 2201-12 (2011).

Section A (public)

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[1] (if available)	Is/Will open access[2] provided to this publication?
				TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES						
1	The Janus faces of CD40 in Cancer	Loskog	Seminars in Immunology	2009 Oct;21(5)	Elsevier Ltd	The Netherlands	2009	301-7.	10.1016/j.smim.2009.07.001.	Yes
2	Suppression of integrin alpha3beta1 in breast cancer cells reduces cyclooxygenase-2 gene expression and inhibits tumourigenesis, invasion and cross-talk to endothelial cells.	Mitchell	Cancer Research	2010 Aug 1;70(15)	AACR	USA	2010	6359-67.	10.1158/0008-5472.CAN-09-4283	Yes
3	CD40 stimulates a "feed-forward" NF-κB-driven molecular pathway that regulates IFN-β expression in carcinoma cells	Moschonas A	J. Immunol	2012 Jun 1;188(11)	The American Association of Immunologists, Inc.	USA	2012	5521-7	10.4049/jimmunol.1200133.	yes
4	BioTextQuest: a web-based biomedical text mining suite for concept discovery.	Papanikolaou	Bioinformatics	2011 Dec 1;27(23)	Oxford University Press	UK	2011	3327-8	10.1093/bioinformatics/btr564	yes
5	Gene-specific factors determine mitotic expression and bookmarking via alternate regulatory elements.	Arampatzi P	Nucleic Acids Res.	2013 Feb 1;41(4)	Oxford University Press	UK	2013	2202-15	10.1093/nar/gks1365.	yes

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[1] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[2] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

6	Programmed death-1 shapes memory phenotype CD8 T cell subsets in a cell-intrinsic manner	Charlton, J. J	J. Immunol	2013 Jun 15;190(12):	The American Association of Immunologists, Inc.	USA	2013	6104-14.	10.4049/jimmunol.1201617	No
7	Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer.	J. M. Penninger	Nature	vol. 468 Nov 4 2010	Nature Publishing Group	UK	2010	98-102	10.1038/nature09387	yes
8	The stress kinase MKK7 couples oncogenic stress to p53 stability and tumor suppression.	J. M. Penninger	Nature Genetics	vol. 43	Nature Publishing Group		2011	212-219	10.1038/ng.767	no
9	. Forward and Reverse Genetics through Derivation of Haploid Mouse Embryonic Stem Cells.	J. M. Penninger	Cell Stem Cells	vol.9, Issue 6, 2 December 2011	CellPress	USA	2011	563–574	http://dx.doi.org/10.1016/j.stem.2011.10.012	no
10	ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation	J. M. Penninger	Nature	vol.487, 26 July 2012	Nature Publishing Group		2012	477–481	10.1038/nature11228	no
11	An immunosurveillance mechanism controls cancer cell ploidy.	J. M. Penninger	Science	vol.237, no. 6102 September 2012	AAAS	USA	2012	1678-1684	10.1126/science.1224922	no
12	The role of macrophage-derived IL-1in induction and maintenance of angiogenesis	Carmi, Y.	J. Immunol.	Volume 183 (2009 Oct) 1	The American Association of Immunologists, Inc.	USA	2009	4705-14	10.4049/jimmunol.0901511	yes
13	Long distance inflammatory and genotoxic impact of cancer in vivo	Bartek	Proceedings of the Natural Academy of Sciences USA	vol. 107 no. 42	National Academy of Sciences.	USA	2010	17861–17862	10.1073/pnas.1013093107	yes

14	Differential release of chromatin-bound IL-1{alpha} discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation.	Cohen, I.	Proc Natl Acad Sci	vol. 107 no. 6February 9, 2010	Washington, DC : National Academy of Sciences	USA	2010	2574–2579	www.pnas.org/content/107/6/2574	yes
15	Replication stress and oxidative damage contribute to aberrant constitutive activation of DNA damage signalling in human gliomas.	Bartkova	Oncogene	2010 Sep 9;29(36)	Nature Publishing Group	UK	2010	5095-102	10.1038/onc.2010.249	No
16	IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation.	Rider, P.	J. Immunol	1;187(9)2011 Nov	The American Association of Immunologists, Inc.	USA	2011	4835-43	10.4049/jimmunol.1102048	yes
17	53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers.	Bouwman	Nature Structural Biology	2010 Jun;17(6)	Nature Publishing Group	USA	2010	688-95.	10.1038/nsmb.1831.	yes
18	The transcription of the alarmin cytokine interleukin-1 alpha is controlled by hypoxia inducible factors 1 and 2 alpha in hypoxic cells.	Rider, P.	Front Immunol	3:290 14 September 2012	Frontiers Media S.A.	Switzerland	2012		10.3389/fimmu.2012.00290.	yes
19	IL-1 is a Major Cytokine that Controls the Balance between Inflammation and Immunity in the Tumor Microenvironment	Apte R.N.	The Inflammatory Milieu of Tumors: Cytokines and Chemokines that Affect Tumor Growth and Metastasis" Edited by Adit Ben Baruch,	2012	Bentham Science Publishers		2012	57-80	10.2174/978160805256111201010057	No

20	Interleukin-1α, a dual-function intracellular cytokine.	Rider, P.	Sem. Immunol	In press			In press	In press	In press	
21	The role of IL-1β in the early tumor cell-induced angiogenic response.	Carmi, Y	J. Immunol	190(7), Apr 1; 2013	The American Association of Immunologists, Inc.	USA	2013	3500-9	10.4049/jimmunol.1202769	No
22	Pleiotropic and differential functions of IL-1α and IL-1β share the tumor microenvironment and affect the outcome of malignancies.	Apte R.N.	"The Tumor Immunoenvironment".	The Tumor Immunoenvironment2013	Spriger	Germany	2013	197-222	10.1007/978-94-007-6217-6_8	yes
23	Non-redundant properties of IL-1α and IL-1β during acute colon inflammation in mice.	BersudskY, M.	Gut	Gut. 2013; Jun 21.	British Society of Gastroenterology (BSG)	UK	2013		10.1136/gutjnl-2012-303329	No
24	Unique Versus Redundant Functions of IL-1α and IL-1β in the Tumor Microenvironment.	Voronov, E	Front Immunol	July 2013 Volume 4 Article 177 | 1	Frontiers Media S.A.	USA	2013		10.3389/fimmu.2013.00177	YES
25	Microenvironment- derived IL-1 and IL-17 interact in the control of lung metastasis	Carmi,Y	J. Immunol	2011 Mar 15;186(6)	The American Association of Immunologists, Inc.	USA	2011	3462-71	10.4049/jimmunol.1002901	Yes+
26	Tpl2 regulates intestinal myofibroblast HGF release to suppress colitis-associated tumorigenesis	George Kollias	Journal of Clinical Investigation	Volume 122, Issue 11, (November 1, 2012)	American Society for Clinical Investigation	USA	2012	4231-42	http://www.jci.org/articles/view/63917	yes

27	Safe TNF-based antitumor therapy following p55TNFR reduction in intestinal epithelium	George Kollias	Journal of Clinical Investigation	123(6), (June 3, 2013)	American Society for Clinical Investigation	USA	2013	2590–2603	http://www.jci.org/articles/view/65624	yes
28	A New Role for Myeloid HO-1 in the Innate to Adaptive Crosstalk and Immune Homeostasis	George Kollias	Advances in experimental medicine and biology	Vol. 780	New York, Plenum Press	USA	2011	101-11	http://link.springer.com/chapter/10.1007%2F978-1-4419-5632-3_9	yes
29	Intestinal epithelial cells as producers but not targets of chronic TNF suffice to cause murine Crohn-like pathology	George Kollias	Proc Natl Acad Sci USA	108(13); March 29	Washington, DC : National Academy of Sciences	USA	2011	5396-401	http://www.pnas.org/content/108/13/5396.long	yes
30	Phosphorylation of the M3/6 dual-specificity phosphatase enhances the activation of JNK by arsenite	George Panayotou	Cellular Signalling	24 (3) March 2012	Elsevier	UK	2012	664-676	http://dx.doi.org/10.1016/j.cellsig.2011.10.015	no
31	Differential regulation of M3/6 (DUSP8) signaling complexes in response to arsenite-induced oxidative stress	George Panayotou	Cellular Signalling	25 (2), February2013	Elsevier	UK	2013	429-438	http://dx.doi.org/10.1016/j.cellsig.2012.11.010	no
32	The Drosophila DUSP Puckered is phosphorylated by JNK and p38 in response to arsenite-induced oxidative stress	George Panayotou	Biochemical and Biophysical Research Communications	418 (2) February 2012	Elsevier	USA	2012	301-306	http://dx.doi.org/10.1016/j.bbrc.2012.01.015	no
33	Myeloid cell expression of the RNA-binding protein HuR protects mice from pathologic inflammation and colorectal carcinogenesis	Dimitris L. Kontoyiannis	Journal of Clinical Investigation	122 (1) January 3, 2012;	American Society for Clinical Investigation	USA	2012	48-61	http://www.jci.org/articles/view/45021	yes
34	HuR controls lung branching morphogenesis and mesenchymal FGF networks	Dimitris L. Kontoyiannis	Dev Biol	354 (2), 15 June 2011	ELSEVIER	USA	2011	267-279	http://www.sciencedirect.com/science/article/pii/S0012160611002132	yes
35	Decoding the functions of post-transcriptional regulators in the determination of inflammatory states: focus on macrophage activation.	Dimitris L. Kontoyiannis	Wiley Interdiscip Rev Syst Biol Med.	4(5) Sep-Oct 2012;	Hoboken, NJ : John Wiley & Sons	USA	2012	509-23	http://onlinelibrary.wiley.com/doi/10.1002/wsbm.1179/abstract;jsessionid=6682BC4725B058A1035280AC711165E4.d04t01	yes

36	Neuroblastoma-targeted nanoparticles entrapping siRNA specifically knockdown ALK	Di Paolo D. et al.	Molecular Therapy	19(6)	Nature Publishing Group	USA	2011	1131-40	10.1038/mt.2011.54	yes
37	Selective Therapeutic Targeting of the Anaplastic Lymphoma Kinase With Liposomal siRNA Induces Apoptosis and Inhibits Angiogenesis in Neuroblastoma	Di Paolo D. et al.	Molecular Therapy	19(12)	Nature Publishing Group	USA	2011	2201-12	10.1038/mt.2011.142	yes
38	Enhanced anti-tumour and anti-angiogenic efficacy of a novel liposomal fenretinide on human neuroblastoma	Di Paolo D. et al.	J Control Release	Volume 170, Issue 3, 28 September 2013	Elsevier		2013	445-451	http://dx.doi.org/10.1016/j.jconrel.2013.06.015	yes
39	DNA damage response, genetic instability and cancer: from mechanistic insights to personalized treatment.	Bartek J	Mol Oncol	Volume 5, Issue 4, August 2011,	Elsevier	Netherlands	2011	303–307	10.1016/j.molonc.2011.07.006	Yes
40	Multicentric breast cancer: clonality and prognostic studies.	Eeles R	Breast Cancer Res Treat	2011 Oct;129(3)	Springer Science	Netherlands	2011	703-16.	10.1007/s10549-010-1230-3	Yes
41	NQO1 expression correlates inversely with NF-kB activation in human breast cancer.	Jamshidi M	Breast Cancer Res Treat	April 2012, Volume 132, Issue 3	Springer Science	Netherlands	2012	955-968	1007/s10549-011-1629-5	Yes
42	Two new CHEK2 germ-line variants detected in breast cancer/sarcoma families negative for BRCA1, BRCA2, and TP53 gene mutations.	Manoukian S	Breast Cancer Res Treat	2011 Nov;130(1)	Springer Science	Netherlands	2011	:207-15.	10.1007/s10549-011-1548-5	Yes
43	Nucleoporin NUP153 guards genome integrity by promoting nuclear import of 53BP1. .	Moudry P	Cell Death Differ	2012 May;19(5):	Nature Publishing Group	UK	2012	798-807	10.1038/cdd.2011.150vvv	Yes
44	Low dose DNA damage and replication stress responses quantified by optimized single-cell imaging analysis	Mistrik	Cell Cycle	8:16, 15 August 2009	Landes Bioscience	USA	2009	2592-2599	10.4161/cc.8.16.9331	Yes
45	Bacterial intoxication evokes cellular senescence with persistent DNA damage and cytokine signaling	Blazkova	Journal of Cellular and Molecular Medicine	Volume 14, Issue 1-2, January-February 2010	John Wiley & Sons, Inc	USA	2010	357–367	10.1111/j.1582-4934.2009.00862.x	Yes

46	Regulation of the PML tumour suppressor in drug-associated senescence of human normal and cancer cells by JAK/STAT-mediated signaling.	Hubackova	Cell cycle	August 1, 2010Volume9:15	Landes Bioscience	USA	2010	3085-3099	10.4161/cc.9.15.12521	Yes
47	Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner and follow expression of p16(ink4a).	Kosar	Cell cycle	2011 Feb 1;10(3)	Landes Bioscience	USA	2011	457-68	10.4161/cc.10.3.14707	Yes
48	Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice.	Michaud M	Science	16 December 2011: Vol. 334 no. 6062	AAAS	USA	2011	1573-1577	10.1126/science.1208347	No
49	Innate or adaptive immunity? The example of natural killer cells	Vivier	Science	2011 Jan 7;331(6013)	AAAS	USA	2011	44-9	10.1126/science.1198687	No
50	Activation of the NLRP3 inflammasome in dendritic cels induces IL-1beta-dependent adaptive immunity	Ghiringhelli	Nature Medicine	2009 Oct;15(10:	Nature Publishing Group		2009	1170-8.	10.1038/nm.2028	No
51	The dendritic cell-tumour cross-talk in cancer.	Ma	Current Opinion in Immunology	Volume 23, Issue 1, February 2011	Elsevier		2011	146–152	http://dx.doi.org/10.1016/j.coi.2010.09.008	Yes
52	Chemotherapy and radiotherapy: cryptic anticancer vaccines.	Ma	Seminars in Immunology	2010 Jun;22(3)	Elsevier		2010	113-24.	doi: 10.1016/j.smim.2010.03.001	Yes
53	How to improve the immunogenicity of chemotherapy and radiotherapy.	Ma	Cancer Metastasis Reviews	2011 Mar;30(1):	Springer Science+Business Media		2011	71–82	DOI 10.1007/s10555-011-9283-2	Yes
54	Cyclophosphamide induces differentiation of Th17 cells in cancer patients.	Viaud	Cancer Research	2011 Feb 1;71(3):	AACR	USA	2011	661-5.	10.1158/0008-5472	Yes

55	Integration of host-related signatures with cancer cell-derived predictors for the optimal management of anticancer chemotherapy.	Zitvogel	Cancer Research	2010 Dec 1;70(23)	AACR	USA	2010	9538-43.	10.1158/0008-5472 CAN-10-1003.	Yes
56	The dendritic cell-like functions of IFN-producing killer dendritic cells reside in the CD11b+ sunset and are licensed by tumour cells	Terme	Cancer Research	2009 Aug 15;69(16)	AACR	USA	2009	6590-7.	10.1158/0008-5472.CAN-08-4473	Yes
57	An inhibitor of cyclin-dependent kinases suppresses TLR signaling and increases the susceptibility of cancer patients to herpes viridae.	Zoubir	Cell Cycle	2011 Jan 1;10(1)	Landes Bioscience	USA	2011	118-26.	**10.4161/cc.10.1.14445**	Yes
58	Alternatively spliced NKp30 isoforms affect the prognosis of gastrointestinal stromal tumours	Delahaye	Nature Medicine	2011 Jun;17(6).	Nature Publishing Group		2011	700-7	10.1038/nm.2366	No
59	Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology	Buonocore, S.	Nature	464	Nature Publishing Group		2010	1371-5	10.1038/nature08949	No
60	Identification of a genetic locus controlling bacteria-driven colitis and associated cancer through effects on innate inflammation	Boulard, O.	J Exp Med	209	The Rockefeller University Press	USA	2012	1309-24	www.jem.org/cgi/doi/10.1084/jem.20120239	After 6 months
61	Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model	Kirchberger, S.	J Exp Med	210	The Rockefeller University Press	USA	2013	917-31	10.1084/jem.20122308	After 6 months
62	Intestinal homeostasis and its breakdown in inflammatory bowel disease.	Maloy KJ	Nature	Vol:474 16 June 2011	Nature Publishing Group		2011	298–306	10.1038/nature10208	
63	Ectonucleotidase CD38 demarcates regulatory, memory-like CD8+ T cells with IFN-γ-mediated suppressor activities	Bahri R, Bollinger A., Bollinger T., Orinska Z. and Bulfone-Paus S.	Plos One	September 17, 2012 (volume7) Issue 9	Public Library of Science	United States	2012		http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0045234	yes

64	Mechanisms and functions of p38 MAPK signalling.	Cuadrado	Biochemical Journal	2010 Aug 1;429(3):	Biochemical Society	UK	2010	403-17.	10.1042/BJ20100323	no
65	Essential role of p18Hamlet/SRCAP-mediated histone H2A.Z chromatin incorporation in muscle differentiation.	Cuadrado	EMBO Journal	2010 June 16; 29(12)	The European Molecular Biology Organization		2010	2014–2025.	10.1038/emboj.2010.85	yes
66	Functional interplay between the DNA damage response kinase ATM and ARF tumour suppressor protein	Velimezi	Nat Cell Biol	Vol 15 , No 8	Macmillan Magazines Ltd	UK	2013	967-77	doi: 10.1038/ncb2795 (PubMed)	no
67	Mutant p53 prolongs NF-κB activation and promotes chronic inflammation and inflammation-associated colorectal cancer protein	Cooks	Cancer Cell	Volume 23, Issue 5, (13 May 2013)	CellPress	USA	2013	23: 634-46	doi: 10.1016/j.ccr.2013.03.022 (PubMed)	no
68	The DNA damage checkpoint precedes activation of ARF in response to escalating oncogenic stress during tumorigenesis	Evangelou	Cell Death Diff		Nature Publishing Group	UK	2013	In press	doi: 10.1038/cdd.2013.76 (PubMed)	no
69	Chronic NF-κB activation delays RasV12-induced premature senescence of human fibroblasts by suppressing the DNA damage checkpoint response	Batsi	Mech Ageing	Volume 130, Issue 7	Elsevier		2009	130: 409-419	10.1016/j.mad.2009.04.002(PubMed)	no
70	p57KIP2: "Kip"ing the cell under control	Pateras	Mol Cancer Res	Vol:7 December 2009	AACR	USA	2009	1902-1919	doi: 10.1158/1541-7786.MCR-09-0317(PubMed)	no
71	Why is p53-inducible gene 3 rarely affected in cancer?	Kotsinas	Oncogene	Vol. 29, 2010	Macmillan Publishers Limited		2010	29: 5220	doi: 10.1038/onc.2010.263(PubMed)	no
72	Oncogene-induced senescence: the bright and dark side of the response	Gorgoulis	Curr Opin Cell Biol	Vol. 22(6),2010	Elsevier		2010	816-27	doi: 10.1016/j.ceb.2010.07.013(PubMed)	no

73	Loss of p14(ARF) confers resistance to heat shock- and oxidative stress-mediated cell death by upregulating beta-catenin	Damalas	Int J Cancer	Volume 128, Issue 8, 2010	Wiley-Blackwell	UK	2010	1989-95	doi: 10.1002/ijc.25510(PubMed)	no
74	The roles of p27(Kip1) and DNA damage signaling in the chemotherapy-induced delayed cell cycle checkpoint	Liontos	J Cell Mol Med	Vol 14, No 9, 2010	Wiley-Blackwell	UK	2010	2264-2267	PMID:20716117[PubMed – as supplied by publisher]	no
75	Increased expression of bFGF is associated with carotid atherosclerotic plaques instability engaging the NF-κB pathway	Sigala	J Cell Mol Med	Volume 14, Issue 9, September 2010	Wiley-Blackwell	UK	2010	pages 2273–2280	10.1111/j.1582-4934.2010.01082.x]	no
76	Oxidized LDL in human carotid plaques is related to symptomatic carotid disease and lesion instability	Sigala	J Vasc Surg	Vol52, Issue3, September 2010	Elsevier		2010	704-13	doi: 10.1016/j.jvs.2010.03.047 (PubMed)	no
77	Cdc6 expression represses E-cadherin transcription and activates adjacent replication origins	Sideridou	J Cell Biol	vol. 195 no. 7	The Rockefeller University Press.	USA	2011	:1123-40	doi: 10.1083/jcb.201108121(PubMed)	no
78	A novel link between oxidative stress and DNA damage response in cance	Kotsinas	Cancer Lett	Volume 327, Issues 1–2	Elsevier		2012	97-102	doi: 10.1016/j.canlet.2011.12.009(PubMed)	no
79	Targeting DNA damage and repair: Embracing the pharmacological era for successful cancer therapy	Aziz	Pharmacol Ther	Volume 133, Issue 3	Elsevier		2012	334-50	doi: 10.1016/j.pharmthera.2011.11.010(PubMed)	no
80	The Tumor Suppressor Gene ARF as a Sensor of Oxidative Stress	Liontos	Curr Mol Med	Vol.12(6) 2012 Jul 1			2012	12:704-15	PMID: 22292438 [PubMed – indexed for MEDLINE] (PubMed)	no
81	Detection of Herplex Simplex Virus-1 and -2 in cardiac myxomas	Pateras	J Bioeng Biotechnol	Volume 2012 (2012), Article ID 823949			2012	2012: 823949	doi: 10.1155/2012/823949(PubMed)	no

82	The single nucleotide polymorphism g.1548A >G (K469E) of the ICAM-1 gene is associated with worse prognosis in non-small cell lung cancer	Thanopoulou	Tumour Biol	October 2012, Volume 33, Issue 5	Springer		2012	1429-36.	doi: 10.1007/s13277-012-0393-4(PubMed)	no
83	The canonical NF-κB pathway differentially protects normal and human tumor cells from ROS-induced DNA damage	Sfikas	Cell Signal	Volume 24, Issue 11, November 2012	Elsevier		2012	2007-2023	doi: 10.1016/j.cellsig.2012.06.010(PubMed)	no
84	Cdc6: a multi-functional molecular switch with critical role in carcinogenesis	Petrakis	Transcription	Vol. 3(3) May 1 2012	Landes Bioscience	USA	2012	124-9	doi: 10.4161/trns.20301(PubMed)	no
85	Toll-like receptor 7 protects from atherosclerosis by constraining "inflammatory" macrophage activation	Salagianni	Circulation	Vol. 126	American Heart Association, Inc	USA	2012	952-62	doi: 10.1161/CIRCULATIONAHA.111.067678(PubMed)	no
86	Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence. A method applicable in cryo-preserved and archival tissues	Georgakopoulou	Aging (Albany NY)	Vol. 5	Impact Journals	USA	2013	37-50	PMID: 23449538 [PubMed – in process] (PubMed)	no
87	TPL2 kinase is a suppressor of lung carcinogenesis	Gkirtzimanaki	Proc Natl Acad Sci USA	Vol. 110	National Academy of Sciences	USA	2013	E1470-9	10.1073/pnas.1215938110(PubMed)	no
88	Lymphotoxin-β receptor ignalling through NF-κB2-RelB pathway reprograms adipocyte precursors as lymph node stromal cells.	J. Caamaño	Immunity	Monthly	Cell Press	USA	2012	Vol 37 Pages 721-34	doi: 10.1016/j.immuni.2012.06.010	No
89	Mesenchymal cell differentiation during lymph node organogenesis.	J. Caamaño	Frontiers in Immunology	Bi monthly	Nature Press		2012	3:381	doi: 10.3389/fimmu.2012.00381	Yes
90	Generation of Lymph Node-Fat Pad Chimeras for the Study of Lymph Node Stromal Cell Origin.	C. Benezech and J. Caamaño	Journal of Visualized Experiments JoVE	Bi monthly			2013	In Press	In Press	Yes

91	Signaling mediated by the NF-κB sub-units NF-κB1, NF-κB2 and c-Rel differentially regulate Helicobacter felis-induced gastric carcinogenesis in C57BL/6 mice.	M Pritchard	Oncogene		Nature publishing Group		2013	In Press	In Press	Yes
92	A Mouse Model of Pathological Small Intestinal Epithelial Cell Apoptosis and Shedding Induced by Lipopolysaccharide	M Pritchard	Disease Models and Mechanisms	In Press	Company of Biologists.	UK	2013	In Press	In Press	Yes
93	NFκB2 p52 has a role in antiviral immunity through IKKε-dependent induction of Sp1 and IL-15	S. Doyle and L. O'Neill	Journal of Biological Chemistry	Aug 30;288(35) Weekly	American Society for Biochemistry and Molecular Biology	USA	2013	25066-75	10.1074/jbc.M113.469122.	Yes
94	Hepatocyte IKK2 protects Mdr2-/- mice from chronic liver failure.	Ehlken H	PLoS ONE	6(10)	Public Library of Science	USA	2011	e25942	10.1371/journal.pone.0025942	Yes
95	The adaptor protein FADD protects epidermal keratinocytes from necroptosis in vivo and prevents skin inflammation.	Bonnet MC	Immunity	Volume 35, Issue 4, 13 October 2011	Cell Press	USA	2011	572-582	10.1016/j.immuni.2011.08.014	No
96	FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation.	Welz PS	Nature	2011 Jul 31;477(7364)	Nature Press		2011	330-4	10.1038/nature10273.	
97	Constitutive IKK2 activation in intestinal epithelial cells induces intestinal tumors in mice.	Vlantis K	J Clin Invest	2011;121(7)	American Society for Clinical Investigation	USA	2011	2781–2793.	10.1172/JCI45349.	Yes
98	TNFR1 signaling in NF-κB-deficient keratinocytes triggers IL-24-dependent psoriasis-like skin inflammation in mice	Snehlata Kumari	Immunity, provisionally accepted							

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99	Stimulating healthy tissue regeneration by targeting the 5-HT2B receptor in chronic liver disease.	Ebrahimkhani MR. Oakley F Joint 1st	Nature Medicine	Vol 17, No 12	Nature Press		2011	1668-73	PMID 22120177	Yes
100	NF-kappaB signalling: Embracing complexity to achieve transition	Chakraborty	Journal of Hepatology	2010 vol. 52	Elsevier	The Netherlands	2010	285–291	10.1016/j.jhep.2009.10.030	Yes
101	Tumor progression locus 2/Cot is required for activation of extracellular regulated kinase in liver injury and toll-like receptor-induced TIMP-1 gene transcription in hepatic stellate cells in mice.	Perugorria MJ.	Hepatology	Vol 57 No 3.	Wiley	Hepatology	2013	1238-49	PMID 23080298	Yes
102	The c-Rel subunit of NF-kB regulates epidermal homeostasis and promotes skin fibrosis in mice.	Fullard N	American Journal of Pathology	Vol 182 No 6	Elsevier	American Journal of Pathology	2013	2109-20	PMID 23562440	Yes
103	The NF-kB subunit c-Rel stimulates cardiac hypertrophy and fibrosis	Gaspar-Pereira S	American Journal of Pathology	Vol 180 No 3	Elsevier	American Journal of Pathology	2012	929-39	PMID 22210479	Yes
104	The Diverse and Complex Roles of NF-kB subunits in Cancer	Perkins ND	Nat Rev Cancer	2012 Jan 19;12(2):	Nature Press		2012	121-32.	10.1038/nrc3204	No
105	NF-κB regulates expression of Polo-like kinase 4	Adeline Ledoux (1st author), Neil Perkins (corresponding author)	Cell Cycle	September 15, 12:18	Landes Bioscience	USA	2013	1–11	PMID: 23974100	yes
106	Mutual protection of ribosomal proteins L5 and L11 from degradation is essential for p53 activation upon ribosomal biogenesis stress	Bursac S.	Proc Natl Acad Sci USA	109	National Academy of Sciences	USA	2012.	20467-72	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3528581/	yes

107	Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis	Golomb L.	Mol Cell	45	CellPress	USA	2012.	222-32	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3270374/	yes
108	Partner exchange: protein–protein interactions in the Raf pathway	Wimmer	Trends in Biochemical Sciences	Vol. 35, No. 12 December 2010	Cell Press	USA	2010	660–668	http://dx.doi.org/10.1016/j.tibs.2010.06.001	yes
109	Raf kinases in cancer–roles and therapeutic opportunities	Maurer	Oncogene	30	Mac Millan	?	2011	3477-88	http://www.nature.com/onc/journal/v30/n32/full/onc2011160a.html	no
110	Mutant p53 prolongs NF-kB activation and promotes chronic inflammation and inflammation-associated colorectal cancer	Cooks T.	Cancer Cell	Volume 23	Cell Press	Boston, USA	2013	634-646	10.1016/j.ccr.2013.03.022.	yes
111	Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis.	Golomb L.	Molecular Cell	Volume 45	Cell Press	Boston, USA	2012	222-232	10.1016/j.molcel.2011.11.022	yes
112	p53 is required for brown adipogenic differentiation and has a protective role against diet-induced obesity.	Molchadsky A	Cell Death & Differentiation	Volume 20	Nature Publishing Group	Rome, Italy	2013	774-783	10.1038/cdd.2013.9	yes
113	Various p53 mutant proteins differently regulate the Ras circuit to induce a cancer-related gene signature.	Solomon H	Journal of Cell Science	Volume 125	The Company of Biologists	Cambridge, UK	2012	3144-3152	10.1242/jcs.099663	yes
114	p53 regulates the Ras circuit to inhibit the expression of a cncer-related gene signature by various molecular pathways.	Buganim	Cancer Research	2010 Mar 15;70(6):	AACR	Philadelphia, USA	2010	2274-84.	10.1158/0008-5472	yes
115	Transcriptional activity of ATF3 in the stromal compartment of tumors promotes cancer progression.	Buganim Y	Carcinogenesis	Volume 32	Oxford Journals	Oxford, UK	2011	1749-1757	10.1093/carcin/bgr203	yes
116	Mutant p53 gain of function is interwoven into the hallmarks of cancer.	Solomon H	The Journal of Pathology	Volume 225	John Wiley & Sons	MA, USA	2011	475-478	10.1002/path.2988	yes

117	p53 regulates the Ras circuit to inhibit the expression of a cancer-related gene signature by various molecular pathways.	Buganim Y	Cancer Research	Volume 70	AACR	Philadelphia, USA	2010	2274-2284	10.1158/0008-5472.CAN-09-2661	yes
118	p53 status in stromal fibroblasts modulates tumor growth in SDF-1-dependent manner.	Addadi Y	Cancer Research	Volume 70	AACR	Philadelphia, USA	2010	9650-9658	10.1158/0008-5472.CAN-10-1146	yes
119	New plays in the p53 theatre	Aylon	Current Opinion in Genetics and Development	2011 Feb;21(1).	Elsevier		2011	86-92	10.1016/j.gde.2010.10.002.	Yes
120	DNA damage response and inflammatory signaling limit the MLL-ENL-induced leukemogenesis in vivo	Takacova S.	Cancer Cell	Vol 21, No 4	CELL PRESS	USA	2012	517-531	PMID: 22516260	Yes
121	TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes	Gudjonsson T.	Cell	Vol 150, No 4	Cell Press	USA	2012	697-709	PMID: 22884692	Yes
122	Autocrine VEGF-VEGFR2-Neuropilin-1 signaling promotes glioma stem-like cell	Hamerlik, P	The Journal of experimental medicine	Vol 209, No 3	Rockefeller University Press	USA	2012	507-520	PMID: 22393126	Yes
123	Macrophages, nitric oxide and microRNAs are associated with DNA damage response pathway and senescence in inflammatory bowel disease	Sohn JJ.	PloS one	Vol 7, No 9	San Francisco, CA : Public Library of Science	USA	2012	N.A.	PMID: 22970173	Yes
124	LEDGF (p75) promotes DNA-end resection and homologous recombination	Daugaard M.	Nature structural & molecular biology	Vol 19, No 8	New York : Nature Pub. Group	USA	2012	803-810	PMID: 22773103	No
125	A high resolution genomic portrait of bladder cancer: correlation between genomic aberrations and the DNA damage response	Schepeler T.	Oncogene	Vol 32, No 31	Nature Publishing Group	England	2013	3577-3586	PMID: 22926521	Yes
126	Interleukin 6 signaling regulates promyelocytic leukemia protein gene expression in human normal and cancer cells.	Hubackova S.	The Journal of biological chemistry	Vol 287, No 32	American Society for Biochemistry and Molecular Biology	USA	2012	26702-14	PMID: 22711534	Yes

127	Thresholds of replication stress signaling in cancer development and treatment	Bartek J.	Nature structural & molecular biology	Vol 19, No 1	Nature Pub. Group	USA	2012	5-7	PMID: 22218289	Yes
128	Centrosome clustering and chromosomal (in)stability: a matter of life and death	Kramer A.	Molecular oncology	Vol 5, No 4	Elsevier	Netherlands	2011	324-3520	PMID: 21646054	Yes
129	Homozygous deficiency of ubiquitin-ligase ring-finger protein RNF168 mimics the radiosensitivity syndrome of ataxia-telangiectasia	Devgan, S.	Cell death and differentiation	Vol 18, No 9	Nature Publishing Group	USA	2011	1500-6	PMID: 21394101	Yes
130	Regulation of stem cell plasticity: mechanisms and relevance to tissue biology and cancer	Strauss R.	Molecular therapy : the journal of the American Society of Gene Therapy	Vol 20, No 5	San Diego, CA : Academic Press	USA	2012	887-97	PMID: 22314288	Yes
131	Utilization of fluorescence in situ hybridization with cytokeratin discriminators in TOP2A assessment of chemotherapy-treated patients with breast cancer	Pierceall WE.	Human pathology	Vol 43, No 9	Philadelphia, PA : WB Saunders	USA	2012	1363-75	PMID: 22204715	Yes
132	Pyrazolo[4,3-d]pyrimidine bioisostere of roscovitine: evaluation of a novel selective inhibitor of cyclin-dependent kinases with antiproliferative activity	Jorda R.	Journal of medicinal chemistry	Vol 54, No 8	American Chemical Society	USA	2011	2980-93	PMID: 21417417	Yes
133	Cep63 recruits Cdk1 to the centrosome: implications for regulation of mitotic entry, centrosome amplification, and genome maintenance	Loffler H.	Cancer research	Vol 71, No 6	American Association for Cancer Research	USA	2011	2129-39	PMID: 21406398	Yes
134	Tethered genes get checked during replication	Lukas J.	Cell	Vol 146, No 2	Cell Press	USA	2011	189-91	PMID: 21784241	Yes
135	MiR-34a expression has an effect for lower risk of metastasis and associates with expression patterns predicting clinical outcome in breast cancer	Peurala H.	PloS one	Vol 6, No 11	San Francisco, CA : Public Library of Science	USA	2011	N.A.	PMID: 22102859	Yes

136	Acetylation dynamics of human nuclear proteins during the ionizing radiation-induced DNA damage response	Bennetzen M.V.	Cell Cycle	Vol 12, No 11	Landes Bioscience	USA	2013	1688-95	PMID: 23656789	No
137	Evaluation of candidate biomarkers to predict cancer cell sensitivity or resistance to PARP-1 inhibitor treatment	Oplustilova L.	Cell Cycle	Vol 11, No 20	Landes Bioscience	USA	2012	3837-50	PMID: 22983061	Yes
138	Small GTPase Rab5 participates in chromosome congression and regulates localization of the centromere-associated protein CENP-F to kinetochores	Serio G.	Proceedings of the National Academy of Sciences of the United States of America	Vol 108, No 42	Washington, DC : National Academy of Sciences	USA	2011	17337-42	PMID: 21987812	Yes
139	Analysis of epithelial and mesenchymal markers in ovarian cancer reveals phenotypic heterogeneity and plasticity	Strauss R.	PloS one	Vol 6, No 1	San Francisco, CA : Public Library of Science	USA	2011	N.A.	PMID: 21264259	Yes
140	Histone Displacement during Nucleotide Excision Repair	Dinant C.	International journal of molecular sciences	Vol 13, No 10	Basel, Switzerland : MDPI	Switzerland	2012	13322-37	PMID: 23202955	Yes
141	Cytokines shape chemotherapy-induced and 'bystander' senescence	Hodny Z.	Aging	Vol 2, No 7	Albany, NY : Impact Journals, LLC	USA	2010	375-6	PMID: 20631421	Yes
142	Long-distance inflammatory and genotoxic impact of cancer in vivo	Bartek J.	Proceedings of the National Academy of Sciences of the United States of America	Vol 107, No 42	Washington, DC : National Academy of Sciences	USA	2010	17861-2	PMID: 20926747	Yes
143	Signal integration by JNK and p38 MAPK pathways in cancer development	Wagner, E.F., Nebreda, A.R	Nat. Rev. Cancer	9	Nature Publishing Group	UK	2009	537-549	10.1038/nrc2694	yes/no

144	Essential role of p18^Hamlet/SRCAP-mediated histone H2A.Z chromatin incorporation in muscle differentiation.	Cuadrado, A., Corrado, N., Perdiguero, E., Lafarga, V., Muñoz-Canoves, P., Nebreda, A.R.	EMBO J.	29	European Molecular Biology Organization	UK	2010	2014-2025	10.1038/emboj.2010.85	yes
145	Mechanisms and functions of p38 MAPK signalling	Cuadrado, A. Nebreda, A.R.	Biochem. J.	429			2010	403-417	10.1042/BJ20100323	
146	Genetic analysis of specific and redundant roles for p38 and p38 MAPKs during mouse development	del Barco Barrantes, I., Coya, J.M., Maina, F., Arthur, J.S.C., Nebreda, A.R.	Proc. Natl. Acad. Sci. USA	108	National Academy of Sciences	USA	2011	12764-12769	www.pnas.org/cgi/doi/10.1073/pnas.1015013108	
147	Roles of p38 MAPKs in invasion and metastasis	del Barco Barrantes, I., Nebreda, A.R.	Biochem. Soc. Trans.	40	Biochemical Society	UK	2012	79-84	10.1042/BST20110676	
148	p38 MAPK signaling	Trempolec, N., Dave-Coll, N., Nebreda, A.R.	Cell	152	Cell Press	USA	2013	656-657	http://dx.doi.org/10.1016/j.cell.2013.01.029	
149	p38 MAPK substrates	Trempolec, N., Dave-Coll, N., Nebreda, A.R.	Cell	152	Cell Press	USA	2013	924-925	http://dx.doi.org/10.1016/j.cell.2013.01.047	

NO.	Type of activities[3]	Main leader	Title	Date/Period	Place	Type of audience[4]	Size of audience	Countries addressed
			TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES					
1	*Conference*	*Voronov, E*	Cancer Immunotherapy and Immunomonitoring (CITIM),	*2009*	Kiev, Ukraine			
2	Conference	*Apte R.N*	The 5th International Congress of The Tumor Microenvironment, Progression, Therapy and Prevention,	*2009*	Versailles, France			
3	Conference	*Voronov, E*	Second European Congress of Immunology,	*2009*	Berlin, Germany.			
4	Conference	*Voronov, E.*	The 14th International Immunology Conference,	*2010*	Kobe, Japan.			
5	Conference	Voronov, E.	Cancer Immunotherapy and Immunomonitoring (CITIM),	2011	Budapest, Hungary			

[3] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[4] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

6	Conference	Apte R.N.	Cancer Immunotherapy and Immunomonitoring (CITIM),	2011	Budapest, Hungary			
7	Conference	Apte R.N.	The IL-1 Family of Cytokines, From Basic Sciences to Clinical Applications,	2011	Clearwater, USA.			
8	Conference	Voronov E.	DKFZ annual meeting,	2012	Jerusalem, Israel			
9	Conference	Voronov E.	Israel Society of Cancer Research (ISCR),	2012	Tel Aviv, Israel.			
10	Conference	Voronov E.	International Conference on Tumor Microenvironment	2012	Suzhou Dushu Lake Conference Center China			
11	Workshop	Voronov E.	FEBS Workshop on: Molecular and Cellular mechanisms in Angiogenesis	2012	Capri, Italy			
12	Conference	Voronov E.	DKFZ annual meeting,	2013	Heidelberg, Germany			
13	Conference	Apte R.N.	Cancer Immunotherapy and Immunomonitoring (CITIM),	2013	Krakow, Poland			

14	Conference	Voronov E.	Cancer Immunotherapy and Immunomonitoring (CITIM),	2013	Krakow, Poland			
15	Conference	Voronov E.	Israel Society of Cancer Research (ISCR),	2013	Beer Sheva, Israel			
16	Conference	George Kollias	Keystone Symposium "The Role of Inflammation during Carcinogenesis"	20-25 May 2012	Dublin, Ireland	Scientific Community	100	International
17	Course	George Kollias	International Course on Laboratory Animal Science	19-30 September 2011	Vari – Athens	Scientific Community	30	International
18	Course	George Kollias	International Course on Laboratory Animal Science	4-15 October 2010	Vari – Athens	Scientific Community	30	International
19	Course	George Kollias	International Course on Laboratory Animal Science	21 September-2 October 2009	Vari – Athens	Scientific Community	30	International
20	Workshop	George Kollias	MUGEN Workshop on Immune Mouse Phenotyping	6-10 October 2009	Athens, Greece	Scientific Community	50	International
21	Conference	George Panayotou	60th conference of HSBMB	20-22 November 2009	Athens-Greece	Scientific Community	700	International
22	Conference	George Panayotou	FEBS Congress	4-9 September 2012	Seville-Spain	Scientific Community	3,000	International
23	Conference	George Panayotou	63rd conference of HSBMB	9-11 November 2012	Heraklion-Greece	Scientific Community	700	International
24	Conference	George Panayotou	63rd conference of HSBMB	9-11 November 2012	Heraklion-Greece	Scientific Community	700	International
25	Conference	Dimitris L. Kontoyiannis	International Conference on Cytokines & Chemokines: Post-transcriptional Regulation.	March 22-24 2010	Saint-Sorlin, France	Scientific Community	150	International

26	Conference	Dimitris L. Kontoyiannis	9th International Conference on Pathways, Networks, and Systems Medicine.	June 10-15, 2011.	Minoa Palace Conference Center - Chania, Crete, Greece.	Scientific Community	100	International
27	Conference	Dimitris L. Kontoyiannis	4th RNA Stability Meeting: RNA turnover & Translation: Biological and Pathological Ramifications	October 17-20, 2010	Montreal, Canada	Scientific	150-200	International
28	Conference	Dimitris L. Kontoyiannis	2nd Inflammation, Cancer & Novel Therapeutics Conference & Summer School	24-28/09/2012	Crete, Greece	Scientific	100-150	European
29	Conference	Dimitris L. Kontoyiannis	Eukaryotic RNA Turnover: From Structural Insights to Diseases	21-24/04/2013	Strasbourg, France	Scientific	*100-150*	European
30	*Publications*	Ponzoni M.	Enhanced anti-tumor efficacy of clinical grade vasculature-targeted liposomal doxorubicin	2008	Clinical Cancer Research		14(22): 7320-7329	
31	*Publications*	Ponzoni M.	Anti-IL-10R antibody improves the therapeutic efficacy of targeted liposomal oligonucleotides.	2009	J Control Release		138(2): 122-7	
32	*Publications*	Ponzoni M.	Recent advances in targeted anti-vasculature therapy: the neuroblastoma model	2009	Curr Drug Targets		10(10): 1021-7	
33	*Publications*	Ponzoni M.	Liposome-Mediated Therapy of Neuroblastoma	2009	Methods in Enzymology		Vol. 465, pp. 225-249	
34	*Publications*	Ponzoni M.	Combined targeting of perivascular and endothelial tumor cells enhances anti-tumor efficacy of liposomal chemotherapy in neuroblastoma	2010	J Control Release		1;145(1): 66-73	

35	*Publications*	Ponzoni M.	Targeted drug delivery and penetration into solid tumors	2012	Med Res Rev		32(5): 1078-91	
36	*Publications*	Ponzoni M.	Novel phage display-derived neuroblastoma-targeting peptides potentiate the effect of drug nanocarriers in preclinical settings	2013	J Control Release		May 25. doi: 10.1016/j.jconrel.2013.04.029	
37	*TV clips*	Ponzoni M. and Perri P.	Gene delivery and gene therapy of cancer	October 2011	Primocanale TV	Media		National
38	*Conferences*	Ponzoni M.	OECI scientific conference titled: "Discovering new worlds in medicine: towards nanoapplication in cancer prevention and treatment". *Oral Presentation* titled:"Drug Delivery: application of liposomal drugs to neuroectodermal cancer treatment	23 May 2008	Palazzo Ducale, Genoa, Italy	Scientific Community	150	National
39	*Workshops*	Pastorino F.	Workshop titled: "Microambiente tumorale". *Oral presentation* titled: "Novel approaches in anti-tumour and vascular targeted therapy".	15 January 2009	Milan, Italy	Scientific Community	50	National

40	*Conferences*	Ponzoni M.	7th NIBIT Meeting. *Oral presentation* titled: "Vasculature-targeted liposomal technology: a novel anti-tumour therapeutic strategy."	1-3 October 2009	Certosa di Pontignano, Siena, Italy	Scientific Community	100	International
41	*Workshops*	Ponzoni M.	Workshop titled: "Microambiente tumorale: ruolo nella progressione neoplastica e nell'immunoregolazione." *Oral presentation* titled: "Vasculature-targeted nano-technology: a novel anti-tumour therapeutic strategy."	14 October 2009	Centro Congressi IST, Genoa, Italy	Scientific Community	50	National
42	*Conferences*	Pastorino F.	SIOPEN Annual General Meeting. *Oral presentation* titled: "Novel Phage Display-Derived Peptides for Tumour- and Vascular-Targeted Therapies against Neuroblastoma".	26th – 28th October 2009	Bambino Gesù Children's Hospital, Rome, Italy	Scientific Community	100	International

43	*Conferences*	Di Paolo D.	AACR-NCI-EORTC International Conference titled: "Molecular targets and Cancer Therapeutics". *Poster* titled: "Effects of a novel liposomal formulaion of fenretinide on human neuroblastoma cell growth, apoptosis and angiogenesis."	15-19 November 2009	Boston, MA, USA	Scientific Community	2000	International
44	*Conferences*	Ponzoni M.	51° SIC scientific conference titled: "Cancer research in the technological post-industrial era." *Oral presentation* titled: "Development of targeted delivery systems for gene-silencing in neuroectodermal tumours	23-26 November 2009	Sesto San Giovanni, Milan, Italy	Scientific Community	200	National
45	*Conferences*	Ponzoni M.	ILS 2009 MEETING titled. "Liposome advances progress in drug and vaccine delivery." *Oral presentation* titled: "Combined targeting of endothelial and perivascular tumour cells enhances antitumour efficacy of liposomal chemotherapy."	*12-15 December 2009*	School of Pharmacy, University of London, London	Scientific Community	300	International
46	*Conferences*	Ponzoni M.	International School of Nano and Biotechnology. Seminar on Nanomedicine and cancer	Gennaio 2010	Vipiteno	Scientific Community	100	International

47	*Conferences*	Perri P.	ANR 2010 Congress titled: " Advances in Neuroblastoma Research". *Oral presentation* titled: "Therapeutic targeting of ALK on neuroblastoma cell by systemic delivery of GD_2-targeted liposomes entrapping siRNA".	21-24 June 2010	Stockholm, Sweden	Scientific Community	400	International
48	*Conferences*	Brignole C.	ANR 2010 Congress titled: " Advances in Neuroblastoma Research". *Poster* titled: "TLR9 expression and functionality in neuroblastoma delineate a novel prognostic marker and therapeutic target".	21-24 June 2010	Stockholm, Sweden	Scientific Community	400	International
49	*Thesis*	Di Paolo D.	PhD in Clinical Pathology. Thesis on antitumour agents and nanotechnolgy	July 2010	University of Genoa, Genoa,Italy	Scientific Community		National
50	*Conferences*	Di Paolo D.	83° SIBS Congress titled. "Ambiente, Salute e Nutrizione". *Oral presentation* titled: "Sviluppo di nuove strategie terapeutiche per la "terapia genica" del neuroblastoma: liposomi e small interference RNA".	21-23 October 2010	Genoa, Italy	Scientific Community	100	National

51	*Conferences*	Ponzoni M.	International Liposomes Research Days & Lipids, Liposomes, & Membrane Biophysics"2010. *Oral presentation* titled: "Tumour-targeted delivery systems for gene-immuno-therapies in neuroctodermal tumours".	4-8 August 2010	Vancouver, CA	Scientific Community	300	International
52	Conferences	Ponzoni M.	23° AICC Congress titled: " Nanotecnologie e veicolazione di farmaci". *Oral presentation* titled: "Targeting vascolare mediato da liposomi come nuovo approcio terapeutico anti-tumorale".	24-26 November 2010	Milan, Italy	Scientific Community	300	National
53	*Workshops*	Ponzoni M. Pastorino F.	Microambiente tumorale: ruolo nella progressione Neoplastica . Workshoop titled: Targeting stromale, tumorale ed antiangiogenico". *Oral presentation* titled: "Targeting tumorale e vascolare come terapia antitumorale"	20 January 2011	Milan, Italy	Scientific Community	50	National
54	*Thesis*	Rossi M.	BSc in Biotecnology Thesis on antitumour agents and nanotechnolgy	April 2011	University of Genoa, Genoa, Italy	Scientific Community		National
55	*Thesis*	Paccagnella M.	BSc in Biotecnology Thesis on antitumour agents and nanotechnolgy	September 2011	University of Genoa, Genoa, Italy	Scientific Community		National

56	*Conferences*	Ponzoni M.	International Conference titled: "Nanodrug delivery from the bench to the patient". *Oral presentation* titled: "Vasculature-targeted nano-technology: a novel anti-tumour therapeutic strategy"	10-13 October 2011	Istituto Superiore Sanità, Rome, Italy	Scientific Community	250	International
57	Conferences	Ponzoni M.	Giornate Umbre di Medicina Molecolare 2011. *Oral presentation* titled. "Tumour and Vascular Targeting Nanoparticles as Therapeutic strategy in Oncology".	21 October 2011	Perugia, Italy	Scientific Community	100	National
58	*Seminar*	Di Paolo D.	Seminar titled: Nuove terapie sperimentali per la cura del Neuroblastoma e loro applicazioni"	15 May 2012	University of Sassari, Sassari, Italy	BSc student, PhD student	50	National
59	*Thesis*	Piaggio F.	BSc in Biotecnology Thesis on antitumour agents and nanotechnolgy	March 2012	University of Genoa, Genoa, Italy	Scientific Community		National
60	*Thesis*	Becherini P.	BSc in Biotecnology Thesis on antitumour agents and nanotechnolgy	March 2012	University of Genoa, Genoa, Italy	Scientific Community		National
61	*Lecture*	Ponzoni M.	University of Coimbra, Lecture title: delivery systems for gene therapy in cancer	September 2012	Coimbra, Portugal	Scientific Community	100	International

62	*Thesis*	Biale C.	PhD Thesis: "RNAinterference and gene delivery systems, mainly Single Walled Carbon Nanotubes"	March 2013	University of Genoa, Genoa, Italy	Scientific Community		National
63	Conference	Powrie, F.	European Congress of Immunology	Jun. 2009	Berlin			
64	Conference	Powrie, F.	British Society of Immunology Congress	Dec. 2009	Liverpool			
65	Conference	Powrie, F.	14th International Congress in Immunology	Aug. 2010	Japan			
66	Conference	Powrie, F.	British Society of Immunology Congress	Dec. 2010	Liverpool			
67	Conference	Powrie, F.	5th ENII EFIS/EJI Immunology Summer School	May 2011	Sardinia			
68	Conference	Powrie, F.	Spetses Summer School	Jun. 2011	Greece			
69	Conference	Powrie, F.	British Society of Immunology Congress	Dec. 2011	Liverpool	Scientific community (higher education, Research) - Industry	400	INTERNATIONAL
70	Conference	Powrie, F.	Keystone symposia: The Biology of Cytokines	Feb. 2012	Colorado			
71	Conference	Powrie, F.	Gordon Research Conference on Immunochemistry & Immunobiology	Jun. 2012	Switzerland			
72	Conference	Powrie, F.	European Congress of Immunology	2012	Glasgow			
73	Oral presentation and abstract Conference		2011 Joint Annual Meeting Italian Society of Immunology, Clinical Immunology and Allergology (SIICA), German Society for immunology (Dgfl)	September 28th-October 1st2011	Conference Center Palariccione, Riccione, Italy	Scientific Community	#800	Europe

74	Poster and abstract Conference		*Death, Danger, Inflammation and Immunity,* 1st Annual Conference of the European Academy of Tumor Immunology (EATI) and 3rd Conference of the European Research Institute for Integrated Cellular Pathology (ERI-ICP)	May 31-June 1, 2012	Institut Pasteur, Paris, France	Scientific Community	250	Europe
75	*Summer School*		*1st Inflamation&Cancer Summer School*	*28 September 2010*	*Crete, Greece*			
76	*EMBO workshop*		*Chromosome structure, damage & repair*	*25 September 2011*	*Sounio, Greece*			
77	*INsPiRe (FP7) workshop*		*Ageing and Cancer cell biology: Convergent and divergent molecular mechanisms*	*27 June 2013*	*Athens, Greece*			
78	*Cold Spring Harbour Laboratory Meeting*		*Eukaryotic DNA Replication & Genome Maintenance*	*9 Sept 2013*	*Cold Spring Harbour Laboratory, USA*			
79	*EMBO workshop*		*The DNA damage response in cell physiology and disease*	*7 Oct 2013*	*Sounio, Greece*			
80	*Conference*	C. Benezech and J. Caamaño	European Congress of Immunology 2012	*5-8/09/2012*	*Glasgow, UK*	Research community and clinical immunologists from EU and USA: students, post doctoral researchers, scientists and medics	*1400*	European countries.
81	Conference	J. Caamaño	European NF-kB Subunit Workshop 2012.	1-3 of October 2012	Giessen, Germany			
82	Conference	J. Caamaño	UK-Ireland NF-kB Meeting	22-24 of April 2013	Liverpool, UK			

83	Conference	J. Caamaño	6th International Kyoto T Cell Conference	3-7 of June 2013	Kyoto, Japan	Higher education and research: students, post doctoral researchers and scientists Mainly Japan and researchers from all over the world	350	Japan
84	Presentation	J. Caamaño	Institute of Immunobiology, Kantonsspital St.Gallen	28/06/2013	St.Gallen, Switzerland	Higher education research and medical community.	50	Swiss
85	Presentation	J. Caamaño	Cambridge Institute for Medical Research, Immunology Seminar Series	14/06/2013	Cambridge, UK	Higher education, research and medical community	70	UK
86	*Conference*	Derek Mann	*European Association for Study of the Liver (EASL) "Signaling in the Liver"*	*26 February 2010*	Amsterdam, Holland	Scientific community	200	EU
87	Conference	Derek Mann	EASL "fibrosis monothematic conference"	17-18 June 20111	Petersberg, Germany	Scientific community	200	EU
88	Workshop	Derek Mann	UK/Ireland NF-kB workshop	June 2011	Maynooth, Ireland	Scientific community	100	UK and Ireland
89	Workshop	Derek Mann	European NF-kB workshop	October 2012	Marburg, Germany	Scientific community	100	EU
90	Symposium	Derek Mann	7th International Symposium for ALPD and Cirrhosis	6-8th Sept 2012	Beijing, China	Scientific community	400	China, Japan, USA and EU.
91	Workshop	Caroline Wilson (Postdoc)	UK/Ireland NF-kB workshop	April 2013	Liverpool, UK	Scientific community	100	UK and Ireland
92	Conference	Neil Perkins	SINAL 2010, Portuguese Signal Transduction Meeting	May 2010	Faro, Portugal	Scientific Community	150	Portugal

93	Conference	Neil Perkins	Brazillian Society of Cell Biology Meeting	July 2010	Sau Paulo, Brazil	Scientific Community	200	Brazil
94	Conference	Neil Perkins	Quantitative analysis of dynamic signaling meeting	September 2010	Liverpool	Scientific Community	100	UK & EU
95	Conference	Neil Perkins	NF-kB: from Immunity to Cancer	November 2010	Paris	Scientific Community	100	EU
96	Conference	Neil Perkins	Quantitative analysis of dynamic signaling meeting	September 2010	Liverpool	Scientific Community	100	UK & EU
97	Conference	Neil Perkins	Transcription and Disease	May 2011	London	Scientific Community	100	UK & EU
98	Workshop	Neil Perkins	UK/Ireland NF-kB workshop	June 2011	Maynooth, Ireland	Scientific Community	100	UK & Ireland
99	Conference	Neil Perkins	Systems Microscopy Centre Opening Symposium	April 2012	Manchester	Scientific Community	100	UK & EU
100	Workshop	Neil Perkins	NF-kB workshop	May 2012	Chapel Hill, NC, USA	Scientific Community	30	USA
101	Conference	Neil Perkins	TENOVUS-SCOTLAND : 30th Anniversary Symposium	June 2012	Glasgow	Scientific Community	200	UK & EU
102	Conference	Neil Perkins	Cell proliferation and cancer	Sept 2012	Rijeka, Croatia	Scientific Community	100	Croatia
103	Workshop	Neil Perkins	European NF-kB subunit workshop	October 2012	Marburg, Germany	Scientific Community	100	EU
104	Workshop	Neil Perkins	UK/Ireland NF-kB workshop	April 2013	Liverpool	Scientific Community	100	UK & Ireland
105	Workshop	Volarevic S.	Advances in Immunology and Cancer Biology	14-18th April 2011.	Istanbul, Turkey	Scientific community	Approx 200	Turkey, Greece, UK
106	Workshop	Volarevic S.	Translational Cancer Research	10-11th November 2011.	Faculty of Medicine, University of Rijeka	Scientific community	approx 60	EU countries, Israel, Croatia
107	Workshop	Volarevic S., Cokaric Brdovcak M, Bursac S	Cell Proliferation in Cancer	13-15th September 2012.	Faculty of Medicine, University of Rijeka	Scientific community	approx 60	EU countries, Israel, Croatia

108	Workshop	Volarevic S. Cokaric Brdovcak M	Live Cell Imaging Microscopy	23-25th May 2013.	Faculty of Medicine, University of Rijeka	Scientific community	approx 50	Denmark, Sweden,Netherlands, Croatia
109	Conference	Volarevic S.	8th Balkan Meeting on Human Genetics	14-17th May 2009.	Cavtat-Dubrovnik, Croatia	Scientific community Scientific communitiy	about 500	Croatia, other Balkan countries, EU, USA
110	Conference	Volarevic S.	EMBO Young Scientists Forum	15-17th June 2009.	Zagreb University, Croatia	Scientific communitiy	about 200	Croatia and the region
111	Conference	Volarevic S.	5th Croatian Oncology Conference	25-28th March 2010.	Dubrovnik, Croatia	Scientific communitiy		Croatia and the region
112	Conference	Volarevic S.	Info day "FP7 Health and IMI Programs"	April 15th 2010.	Ruđer Bošković Institute, Zagreb, Croatia	Scientific community		Croatia and the region
113	Conference	Volarevic S.	EMBO Practical Course " Anatomy and Embriology of the Mouse"	11-19th September 2010.	Split, Croatia	Scientific community		Croatia, other Balkan countries, EU, USA
114	Conference	Volarevic S.	Croatian Congress of Pharmacology	15-18th September 2010.	Opatija, Croatia	Scientific community		
115	Conference	Volarevic S.	1st Inflammation and Cancer Summer School	28-30th September 2010.	Crete, Greece	Scientific communitiy		
116	Conference	Volarevic S.	TransMedRi kick-off meeting (FP7 REGPOT)	July 20th 2010..	Faculty of Medicine, University of Rijeka	Scientific communitiy		
117	Lecture	Volarevic S.	10th Congress of the Croatian Society of Biochemistry and Molecular Biology	15-18th September 2010.	Opatija, Croatia	Scientific communitiy		
118	Lecture	Volarevic S.	5th Central European Conference "Chemistry towards Biology"	8-11th September 2010.	Primošten, Croatia	Scientific communitiy		

119	Lecture	Volarevic S.	391st Coloqium of Croatian Society of Biochemistry and Molecular Biology	March 1st 2010.	Rijeka, Croatia	Scientific community		
120	Lecture	Volarevic S.	"From Ruđer Bošković to Today"	May 31st-June 1st 2011.	Dubrovnik, Croatia	Scientific community	approx 100	Germany, Switzerland, Italy, Croatia, Israel, France, Canada, USA, Singapore, Korea
121	Lecture	Volarevic S.	"Regulation of the p53 tumor suppressor by ribosomal proteins"	June 4th-8th 2011.	Columbia University, New York, USA	Scientific community	approx 70	
122	Lecture	Volarevic S.	" Ribosomal protein deficiencies and protein activation "	January 18th-22nd 2011.	Rehovot, Israel	Scientific community	approx 50	
123	Lecture	Volarevic S	"Ribosome biogenesis stress and p53 activation"	November 23rd 2012.	The University of Geneva, Switzerland	Scientific community	approx 80	
124	Lecture	Volarevic S.	Inflammation, Cancer and Novel Therapeutics Conference and Summer School : "Defects in ribosome biogenesis and disease"	24-27th September 2012.	Malia, Crete, Greece	Scientific community	about 100	many European countries
125	Lecture	Volarevic S.	3rd FEBS meeting "From Molecules to Life and Back": "Ribosomal proteins and p53 regulation"	June 16th 2012.		Scientific community	about 300	
126	Lecture	Volarevic S.	Latest advances in cancer research and molecular biology	March 18th and April12th 2013.	Rijeka, Croatia	Other		
127	Lecture	Volarevic S.	Presentation of INFLA-CARE project: League Against Cancer	February 15th 2013.	Rijeka, Croatia	Other		
128	Training	Grabusic K., Orsolic I.	Training in DNA sequencing and basic principles of bioinformatics	December 15th-19th 2009.	University of Oslo, Department of Oral Biology, Oslo, Norway			

129	Training	Vukelic I, Bursac S, Grabusic K.	1st Inflammation &Cancer Summer School	September 28-30th 2010.	Crete, Greece			
130	Training	Bursac S.	Training in various molecular biology techniques	April10th-17th 2011.	FORTH, Crete, Greece			
131	Training	Cokaric Brdovcak M.	Training in polysome profile analysis	January 15th-25th 2011.	Hebrew University of Jerusalem, Israel			
132	Training	Vukelic I.	INFLA-CARE annual meeting	October 10th-11th 2011.	Athens, Greece			
133	Training	Bursac S, Cokaric Brdovcak M.	INFLA-CARE annual meeting & 2nd Inflammation, Cancer and Novel Therapeutics Conference & Summer School	September 22nd-27th 2012.	Malia, Crete, Greece			
134	Training	Brdovcak Cokaric M.	Pathohistological analysis of the liver	April 7th-14th 2013.	National Kapodistrian University of Athens, Greece			
135	Interview	Volarevic S.	http://novine.novilist.hr	April 11th 2008.	NOVI LIST, Rijeka, Croatia	Media		
136	Interview	Volarevic S.	http://novine.novilist.hr	June 15th 2009.	NOVI LIST, Rijeka, Croatia	Media		
137	Interview	Volarevic S.	Talk about research potential of Croatia, his current research interests and projects including INFLA-CARE	June 18th 2010.	Talk show "Lica nacije", broadcasted on Croatian National Television (HRT)	Media		
138	Interview	Volarevic S.	Talk about recent progress in cancer research	October 2010.	broadcasted on Croatian National Television (HRT)	Media		
139	Conference	Raguz	World Immune Regulation Meeting "Innate and Adaptive Immune Response and Role of Tissues in Immune Regulation"	Mar 24 – 27, 2011	Davos, Switzerland	Scientific Community	>100	World-wide

140	Conference	Raguz	CSHL Asia Conference "Frontiers of Immunology in Health and Diseases"	Sep 03 -07, 2012	Shanghai, China	Scientific Community	>100	World-wide
141	Research summit	Raguz	Research Summit "Signals & Enzymes"	Dec 9 – 12, 2012	Graz, Austria	Scientific Community	120	A
142	PhD retreat	Raguz	PhD retreat "Signaling Cancer". Vienna, Austria	Dec 10 -12, 2010	Vienna Austria	Scientific Community	120	A
143	Conference	Jeric	CSHL Conference "Models of Cancer"	Aug 14 – 18, 2012	Cold Spring Harbor – USA	Scientific Community	>100	World-wide
144	Conference	Jeric	CSHL Asia Conference "Liver and Metabolic Disease and Cancer"	May 21 – 25, 2012	Shanghai, China	Scientific Community	>100	World-wide
145	Research summit	Jeric	Research Summit "Signals & Enzymes"	Dec 9 – 12, 2012	Graz, Austria	Scientific Community	120	A
146	PhD retreat	Jeric	PhD retreat "Signaling Cancer". Vienna, Austria	Dec 10 -12, 2010	Vienna Austria	Scientific Community	120	A
147	Conference	Cavallo	The EMBO Meeting 2009	Aug 29 – Sep 01, 2009	Amsterdam, NL	Scientific Community	>100	World-wide
148	*Conference*	*Oren*	*Mutant p53 workshop*	*5.2011*	*Rome, Italy*	*Research*	*160*	*International*
149	*Conference*	*Oren*	*Mutant p53 workshop*	*6.2013*	*Toronto, Canada*	*Research*	*150*	*International*
150	*Conference*	*Oren*	*FAMRI Annual Symposium*	*5.2013*	*Miami, Florida*	*Research + lay*	*300*	*International*
151	*Conference*	*Oren*	*European Cancer Congress*	*10.2013*	*Amsterdam*	*Research + medical*	*10,000*	*Europe + international*
152	*Conference*	*Oren*	*AACR Annual Meeting*	*4.2013*	*Washington DC*	*Research + medical*	*500*	*International*
153	*Presentation*	*Oren*	*Lecture*	*5.2013*	*Houston, Texas*	*Research*	*300*	*USA*
154	*Presentation*	*Oren*	*Lecture*	*5.2011*	*Bar-Ilan University, Israel*	*Research*	*100*	*Israel*
155	*Presentation*	*Oren*	*Lecture*	*10.2011*	*Tel Aviv University, Israel*	*Research*	*100*	*Israel*
156	*Presentation*	*Oren*	*Lecture*	*11.2011*	*Karolinska Institute*	*Research*	*60*	*Sweden*

157	*Conference*	*Rotter*	*Lecture*	*5.2013*	*Italian National Cancer Institute Regina Elena, Rome, Italy*	*Research*	*100*	*International*
158	*Conference*	*Rotter*	*Lecture*	*5.2013*	*FEBS Editorial Board Meeting*	*Heidelberg, Germany*	*100*	*International*
159	*Conference*	*Rotter*	*12th FAMRI symposium*	*5.2013*	*Miami, Florida*	*Research + lay*	*300*	*International*
160	*Conference*	*Rotter*	*Frontiers in Cancer Research and Therapy*	3.2013	*Karolinska Institutet, Sweden*	*Research*	100	*International*
161	*Conference*	*Rotter*	*Lecture*	3.2013	*Switzerland*	*Research*	100	*International*
162	*Conference*	*Rotter*	*HDIR-2; second meeting: From Bench to Clinic*	*11.2012*	*Croatia*	*Research*	100	*International*
163	*Conference*	*Rotter*	*ESF-Exploratory Workshop on the "Physics of Cancer"*	*9.2012*	*Verenna, Italy*	*Research*	100	*International*
164	Participation of Dr L.Zacharia to INFA-CARE Kick-off Meeting	Eliopoulos	INFA-CARE Kick-off Meeting in Cologne	6-7 April 2009	Cologne, Germany	*Scientific*	*50*	EC
165	Participation of Dr M.Denis to the 1st INFLA-CARE Annual meeting	Eliopoulos	1st INFLA-CARE Annual meeting	15-17 November 2009	Vienna, Austria	*Scientific*	*50*	EC
166	Participation of Dr M.Denis to the 2nd INFLA-CARE Annual meeting	Eliopoulos	2nd INFLA-CARE Annual meeting	26-27 September 2010	Crete, Greece	*Scientific*	*50*	EC
167	Participation of Dr M.Denis to SADEL meeting	Denis	SADEL meeting	12-14 January 2011	Milano, Italy	*Scientific*	20	EC
168	Participation of Dr M.Denis to INFLA-CARE 3rd Annual Meeting	Eliopoulos	3rd INFLA-CARE Annual Meeting	10-11 October 2011	Athens, Greece	*Scientific*	*50*	EC
169	Participation of K. Kranidioti to the 4th INFLACARE Annual meeting	Eliopoulos	4th INFLACARE Annual meeting	22-24 September 2012	Crete, Greece	*Scientific*	*50*	EC
170	Workshop	A.R.Nebreda	INFLA-CARE 2nd year meeting	27 September 2010	Crete, Greece	Scientific Community	100	*EU & Israel*
171	Summer School	A.R.Nebreda	Inflammation & Cancer	28 September 2010	Crete, Greece	Scientific Community	100	*International*

172	Symposium	A.R.Nebreda	South American Spring Symposium in Signal Transduction and Molecular Medicine	25 October 2010	Cordoba, Argentina	Scientific Community	
173	Workshop	A.R.Nebreda	Genomic Instability Workshop	20 November 2010	Milan, Italy	Scientific Community	
174	Symposium	A.R.Nebreda	Signaling Cancer Symposium	10 December 2010	Vienna, Austria	Scientific Community	
175	Conference	A.R.Nebreda	Signaling by p38 MAPKs in cancer	15 February 2011	London, UK	Scientific Community	
176	Conference	A.R.Nebreda	Signaling by p38 MAPKs in cancer	3 May 2011	Barcelona, Spain	Scientific Community	
177	Conference	A.R.Nebreda	Signaling by p38 MAPKs	6 May 2011	Athens, Greece	Scientific Community	
178	Conference	A.R.Nebreda	Cell Regulation by p38 MAP kinases	3 June 2011	Cordoba, Spain	Scientific Community	
179	Symposium	A.R.Nebreda	Signalling 2011: A Biochemical Society Centenary Celebration Symposium	9 June 2011	Edinburgh, UK	Scientific Community	
180	Symposium	A.R.Nebreda	International Symposium on Cell Division	29 June 2011	Hakone, Japan	Scientific Community	
181	Conference	A.R.Nebreda	The role of p38 MAPKs in tumorigenesis	4 July 2011	Tokyo, Japan	Scientific Community	
182	Symposium	A.R.Nebreda	XXXIV Congreso de la Sociedad Española de Bioquímica y Biología Molecular	6 September 2011	Barcelona, Spain	Scientific Community	
183	Conference	A.R.Nebreda	Regulation of tumorigenesis by p38 MAPKs	10 November 2011	Copenhagen, Denmark	Scientific Community	
184	Conference	A.R.Nebreda	Cell regulation by p38 MAPKs	23 January 2012	Barcelona, Spain	Scientific Community	
185	Symposium	A.R.Nebreda	Cell Signaling and Regulation	22 March 2012	San Diego, California, USA	Scientific Community	
186	Symposium	A.R.Nebreda	Protein Phosphorylation in Signal Transduction and Disease	26 June 2012	Dundee, UK	Scientific Community	

187	Workshop	A.R.Nebreda	FASEB Science Research Conference	24 July 2012	Snowmass Village, Colorado, USA	Scientific Community		
188	Symposium	A.R.Nebreda	The EMBO meeting 2012	24 September 2012	Nice, France	Scientific Community		
189	Symposium	A.R.Nebreda	Antitumoral Targets Upstream and Downstream of Ras GTP-ases	9 October 2012	Santander, Spain	Scientific Community		
190	Symposium	A.R.Nebreda	Biochemistry, Biology and Pathology of MAP kinases	15 October 2012	Jerusalem, Israel.	Scientific Community		
191	Conference	A.R.Nebreda	Signal integration by p38 MAPKs	20 November 2012	Oxford, UK	Scientific Community		
192	Symposium	A.R.Nebreda	3rd cell cycle and cancer meeting	5 April 2013	Montpellier, France	Scientific Community		
193	Conference	A.R.Nebreda	Signal integration by p38 MAPKs	15 April 2013	Uppsala, Sweden	Scientific Community		
194	*Conference*	Bartek J.	*European Conference on Nanotechnologies*	*26 February 2010*				
195	Conference	Bartek J.	American Association for Cancer Research Annual Meeting	2-6April, 2011	Florida, USA	Scientific community, Research, Industry	Over 15000	
196	Conference	Bartek J.	American Association for Cancer Research Annual Meeting	6-10 April, 2013	Washington, USA	Scientific community, Research, Industry	Over 15000	
197	Conference	Bartek J.	Cambridge Healthtech Institute's Ninth Annual HIGH CONTENT ANALYSIS	10-13 January, 2012	San Francisco, USA	Scientific community, Research, Industry	Over 5000	
198	Conference	Bartek J.	12th International Workshop on Radiation Damage to DNA	2-6 June, 2012	Prague, Czech Republic	Scientific community, Research, Industry	Over 200	
199	Conference	Bartek J.	15th Danish Cancer Society Symposium	19-21 October, 2009	Copenhagen, Denmark	Scientific community, Research, Industry	Over 200	

200	Conference	Manolis Pasparakis	International TNF conference	July 7 - 10, 2013	Quebec city, Canada	Scientific Community		
201	Conference	Manolis Pasparakis	Annual Meeting of the Japanese Society of Immunology	December 4-7, 2013	Kobe, Japan	Scientific Community		
202	Conference	Manolis Pasparakis	Epithelial meeting of the German Society of Cell Biology	November 7-9, 2013	Leibzig, Germany	Scientific Community		

Section B (Confidential[5] or public: confidential information to be marked clearly)
Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[6]:	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Application reference(s) (e.g. EP123456)**	**Subject or title of application**	**Applicant (s) (as on the application)**
Patent	Yes		PCT/IB2011/054571	Lipid vectors delivery gene silencers	Perri. P., Pagnan G., Pastorino F. and Ponzoni M.
Patent	NO		EP 2419503	Method of production of synchronized adherently growing cell lines and device for carrying out said method	Martin Mistríik, Jiri Bartek

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[5] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[6] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A	**General Information** *(completed automatically when Grant Agreement number is entered.*	
Grant Agreement Number:		**223151**
Title of Project:		**INFLACARE**
Name and Title of Coordinator:		**Professor Aristides G. Eliopoulos**

B	**Ethics**	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*		*Yes*
2. Please indicate whether your project involved any of the following issues (tick box) :		*YES*
RESEARCH ON HUMANS		
• Did the project involve children?		
• Did the project involve patients?		Yes
• Did the project involve persons not able to give consent?		
• Did the project involve adult healthy volunteers?		
• Did the project involve Human genetic material?		Yes
• Did the project involve Human biological samples?		Yes
• Did the project involve Human data collection?		
RESEARCH ON HUMAN EMBRYO/FOETUS		
• Did the project involve Human Embryos?		
• Did the project involve Human Foetal Tissue / Cells?		
• Did the project involve Human Embryonic Stem Cells (hESCs)?		
• Did the project on human Embryonic Stem Cells involve cells in culture?		
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?		
PRIVACY		
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?		
• Did the project involve tracking the location or observation of people?		
RESEARCH ON ANIMALS		
• Did the project involve research on animals?		yes
• Were those animals transgenic small laboratory animals?		yes

• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	yes
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator		1
Work package leaders	3	6
Experienced researchers (i.e. PhD holders)	29	22
PhD Students	11	7
Other	12	3

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	
Of which, indicate the number of men:	

D   Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project?**	X ⦿	Yes No

6.   Which of the following actions did you carry out and how effective were they?

		Not at all effective			Very effective	
X	Design and implement an equal opportunity policy	○	○	○	●	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
X	Actions to improve work-life balance	○	○	●	○	○
○	Other:					

7.   Was there a gender dimension associated with the research content – **i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?**

     o   Yes- please specify

     ●   No

E   Synergies with Science Education

8.   Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

     X   Yes- please specify
The project was discussed at the "Science Days" at Ben Gurion University to the general public and also in various forums of students who were interested in pursuing advanced degrees and looked for a lab in which to perform their M.Sc or Ph.D studies. Fleming Institute and FORTH (P1) received high school student visits and summer internships for undergraduate students from the nearby Universities of Athens and Crete, respectively. University of Birmingham Participated in science fairs and in open days at the Medical School. Universities of Rijeka and Oxford received high school pupils on work experience or short visits.

     ○   No

9.   Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

     X   Yes- please specify
Website

     ○   No

F   Interdisciplinarity

10.   Which disciplines (see list below) are involved in your project?

     ○   Main discipline[7]:
     3
     1.5 Biological sciences
     1.5   Biological sciences
     Natural and medical sciences
     Biological sciences
     1.5

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[7] Insert number from list below (Frascati Manual).

	1.5 Biological sciences 1.5		
O	Associated discipline[7]: 3.1 3.1	O	Associated discipline[7]: 3.2

G	**Engaging with Civil society and policy makers**		

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	X O	Yes No

11b **If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?**

O	No
X	Yes- in determining what research should be performed
X	Yes - in implementing the research
X	Yes, in communicating /disseminating / using the results of the project

11c	**In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?**	O X	Yes No

12. **Did you engage with government / public bodies or policy makers (including international organisations)**

O	No
X	Yes- in framing the research agenda
O	Yes - in implementing the research agenda
O	Yes, in communicating /disseminating / using the results of the project

13a **Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?**

	Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
X	Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
O	No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade			
Customs		Fisheries and Maritime Affairs		**Public Health**	X
Development Economic and Monetary Affairs		Food Safety		Regional Policy	X
Education, Training, Youth	X	Foreign and Security Policy			
Employment and Social Affairs		Fraud		**Research and Innovation**	
		Humanitarian aid		Space	
				Taxation	
				Transport	

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13c	**If Yes, at which level?**
	O Local / regional levels
	X National level
	O European level
	X International level

H   Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**149**

To how many of these is open access[8] provided?	**89**
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	

❑ publisher's licensing agreement would not permit publishing in a repository
❑ no suitable repository available
X no suitable open access journal available
X no funds available to publish in an open access journal
❑ lack of time and resources
❑ lack of information on open access
❑ other[9]: ……………

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*	2	
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	
	Registered design	1
	Other	

17. How many spin-off companies were created / are planned as a direct result of the project?	
Indicate the approximate number of additional jobs in these companies:	

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

X	Increase in employment, or	X	In small & medium-sized enterprises
X	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,		None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	

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[8] Open Access is defined as free of charge access for anyone via Internet.
[9] For instance: classification for security project.

Difficult to estimate / not possible to quantify	❑

I   Media and Communication to the general public

20.	**As part of the project, were any of the beneficiaries professionals in communication or media relations?**
	Yes X No

21.	**As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?**
	X Yes ◯ No

22	**Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?**

x	Press Release	X	Coverage in specialist press
x	Media briefing	❑	Coverage in general (non-specialist) press
x	TV coverage / report	X	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
❑	Brochures / posters / flyers	X	Website for the general public / internet
❑	DVD / Film / Multimedia	X	Event targeting general public (festival, conference, exhibition, science café)

23	**In which languages are the information products for the general public produced?**

x	Language of the coordinator	❑	ENGLISH
			FRENCH,
x	Other language(s)		GREEK
			ITALIAN
			SPANISH