**DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO**

Proposta di assegnazione di una borsa di Dottorato

**TITLE**: “TARGETING BCL-2 PROTEIN IN COMBINATION WITH IMMUNOTHERAPY TO IMPROVE MELANOMA TREATMENT”.

**TITOLO**: “L’INIBIZIONE DI BCL-2 COME NUOVA STRATEGIA TERAPEUTICA PER INCREMENTARE L’EFFETTO DELL’IMMUNOTERAPIA NEL TRATTAMENTO DEL MELANOMA”

**PROPOSED SUPERVISOR**: Donatella Del Bufalo, UOSD Preclinical Models and New

Therapeutic Agents Unit, IRCCS Regina Elena National Cancer Institute, Rome.

(donatella.delbufalo@ifo.gov.it)

**DESCRIZIONE DELLA RICERCA** (max 2 pagine) Background

Metastatic melanoma is one of the most highly mutated, molecular heterogeneous and lethal type of cancer. The most prominent genetic alterations driving melanomagenesis result in the constitutive activation of the mitogen-activated protein kinase (MAPK) pathway, with BRAF and NRAS hot-spot mutations accounting for about 50% and 30% of all melanoma cases, respectively (1,2). To date, the standard-of-care for BRAF mutant metastatic melanoma includes the BRAF and MEK inhibitors (BRAFi/MEKi) combination therapy or immunotherapy with anti-PD-1, anti–CTLA-4 antibodies, or the combination of the two immune checkpoint inhibitors (ICI) (3). Moreover, therapeutic options are still limited for patients without BRAF mutations and most patients treated with BRAFi/MEKi develop resistance by multiple mechanisms that, in the majority of cases, results in the re-activation of MAPK pathway or in the up-regulation of other pro-survival signaling pathways (4). Therefore, the development of new therapeutic combinations is an urgent need to improve the outcome of patients. In this context, the bcl-2 oncogenic network is one of the most crucial regulators of melanoma cell apoptosis (5) involved in therapeutic resistance (6-8). Previous studies conducted by the hosting laboratory demonstrated that, in addition to its canonical anti-apoptotic role, bcl-2 promotes tumor progression-associated in vitro properties, in vivo tumor growth, angiogenesis and metastatization of melanoma and other tumor histotypes (9-12). Importantly, in a recent paper we demonstrated that Bcl-2 promotes a pro-inflammatory and immunosuppressive tumor niche fueled by M2 tumor associated macrophages (13). Due to their multiple functions in cancer, bcl-2 protein has become interesting target for anti-cancer drugs. To date, different BH3 mimetics against the bcl-2 anti-apoptotic members have been developed and used in hematological malignancies. Among them, ABT-199 (Venetoclax), a first-in-class cancer drug that interacts with the cellular apoptotic machinery promoting apoptosis, is a bcl-2 specific inhibitor that has been recently approved by FDA for adult patients with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma. On the other hand, the efficacy of bcl-2 inhibitors in solid tumors has not been deeply investigated yet. A phase I/II clinical trial is ongoing to test the efficacy of ABT-263 in combination with BRAFi/MEKi in metastatic melanoma (ClinicalTrials.gov NCT01989585).

Objectives

The relevance of bcl-2 protein expression in melanoma has been extensively investigated for its role in the acquisition of drug resistance, but also for its effect on tumor growth and aggressiveness. To date, however, there are few indications on the ability of BH3 inhibitors to predict ICI treatment response or to synergize with ICI to improve melanoma patients outcome.

The objectives proposed in this project could put the rationale for the use of bcl-2 as a therapeutic target in melanoma.

References

1. The Cancer Genome Atlas Network. Genomic Classification of Cutaneous Melanoma. Cell. 2015.

2. Palmieri G et al. Molecular Pathways in Melanomagenesis: What We Learned from Next-

Generation Sequencing Approaches. Curr Oncol Rep. 2018.

3. Luke JJ et al. Targeted agents and immunotherapies: optimizing outcomes in melanoma. Nat Rev

Clin Oncol. 2017.

4. Lito P et al. Tumor adaptation and resistance to RAF inhibitors. Nat Med. 2013.

5. Hartman L et al. Anti-apoptotic proteins on guard of melanoma cell survival Cancer Letter. 2013.

6. Croce CM and Reed JC. Finally, An Apoptosis-Targeting Therapeutic for Cancer. Cancer

Research. 2016.

7. Rohrbeck L et al. Hepatocyte growth factor renders BRAF mutant human melanoma cell lines resistant to PLX4032 by downregulating the pro-apoptotic BH3-only proteins PUMA and BIM. Cell Death and Differentiation. 2016.

8. Lee EF et al. BCL-XL and MCL-1 are the key BCL-2 family proteins in melanoma cell survival.

Cell Death Dis. 2019.

9. Trisciuoglio D et al. Bcl-2 overexpression in melanoma cells increases tumor progression-

associated properties and in vivo tumor growth. J Cell Physiol. 2005.

10. Gabellini C et al BH4 domain of bcl-2 protein is required for its proangiogenic function under

hypoxic condition. Carcinogenesis. 2013.

11. Trisciuoglio D et al. Removal of the BH4 domain from Bcl-2 protein triggers an autophagic

process that impairs tumor growth. Neoplasia. 2013.

12. Gabellini C et al. Non-canonical roles of Bcl-2 and Bcl-xL proteins: relevance of BH4 domain.

Carcinogenesis. 2017.

13. Di Martile M et al. Melanoma-specific bcl-2 promotes a protumoral M2-like phenotype by

tumor-associated macrophages. J Immunother Cancer. 2020.

**LAVORI PUBBLICATI NEGLI ULTIMI 5 ANNI DAL DOCENTE GUIDA (2017-2022)**

1. Valentini E, Simona D’Aguanno S, Di Martile M, Montesano C, Ferraresi V, Patsilinakos A, Sabatino M, Antonini L, Chiacchiarini M, Valente S, Mai A, Colotti G, Ragno R, Trisciuoglio D, Del Bufalo D. “Targeting the anti-apoptotic Bcl-2 family proteins: machine learning virtual screening and biological evaluation of new small molecules” Theranostic, doi:10.7150/thno.64233, in press.

2. Del Bufalo D, Di Martile M, Valentini E, Manni I, Masi I, D’Amore A, Filippini A, Nicoletti C, Zaccarini M, Cota C, Castro MV, Quezada MJ, Rosanò L, Lopez-Bergami P, D’Aguanno S. “Bcl2L10 increases aggressive features of melanoma cells” Explor Target Antitumor Ther. 2022;3:11–26 DOI: https://doi.org/10.37349/etat.2022.00068.

3. Ricci A, Gallorini M, Del Bufalo D, Cataldi A, D'Agostino I, Carradori S, Zara S. Negative modulation of the angiogenic cascade induced by allosteric kinesin Eg5 inhibitors in a gastric adenocarcinoma in vitro model. Molecules. 2022 Jan 31;27(3):957. doi: 10.3390/molecules27030957. PMID: 35164221.

4. Carè A, Del Bufalo D, Facchiano A. Editorial on Special Issue "Advances and Novel Treatment Options in Metastatic Melanoma". Cancers (Basel). 2022 Jan 29;14(3):707. doi: 10.3390/cancers14030707. PMID: 35158974; PMCID: PMC8833463.

5. Nowak RP, Tumber A, Hendrix E, Salik Zeya Ansari M, Sabatino M, Antonini L, Andrijes R, Salah E, Mautone N, Pellegrini FR, Simelis K, Kawamura A, Johansson C, Passeri D, Pellicciari R, Ciogli A, Del Bufalo D, Ragno R, Coleman ML, Trisciuoglio D, Mai A, Oppermann U, Schofield CJ, and Dante Rotili. First-in-Class Inhibitors of the Ribosomal Oxygenase MINA53 J. Med. Chem. 2021, 64, 17031−17050

6. Guerriero C, Matera C, Del Bufalo D, De Amici M, Conti L, Dallanoce C and Tata AM. The Combined Treatment with Chemotherapeutic Agents and the Dualsteric Muscarinic Agonist Iper-8-Naphthalimide Affects Drug Resistance in Glioblastoma Stem Cells. Resistance in Glioblastoma Stem Cells. Cells 2021,

10, 1877. doi.org/10.3390/cells10081877.

7. Di Martile M, Garzoli S, Sabatino M, Valentini E, D'Aguanno S, Ragno R, Del Bufalo D. Antitumor effect of Melaleuca alternifolia essential oil and its main component terpinen-4-ol in combination with target therapy in melanoma models. Cell Death Discov. 2021 May 31;7(1):127. doi: 10.1038/s41420-021-

00510-3.

8. D’Aguanno S, Mallone F, Marenco M, Del Bufalo D, Moramarco A. Hypoxia-dependent drivers of melanoma progression. J Exp Clin Cancer, in press. J Exp Clin Cancer Res. 2021 May 8;40(1):159. doi:

10.1186/s13046-021-01926-6.

9. Valentini E, Di Martile M, Del Bufalo D, Simona D’Aguanno. Semaphorins and their receptors: focus on the crosstalk between melanoma and hypoxia. J Exp Clin Cancer Res. 2021 Apr 15;40(1):131. doi:

10.1186/s13046-021-01929-3.

10. Lucianò AM, Perez-Oliva AB, Mulero V, Del Bufalo D. Bcl-xL: A Focus on Melanoma Pathobiology.

Int J Mol Sci. 2021 Mar 9;22(5):2777. doi: 10.3390/ijms22052777.

11. Trisciuoglio D and Del Bufalo D. New insights on the role of anti-apoptotic members of Bcl-2 family in melanoma progression and therapy. In stampa su Drug Discovery Today. 2021 Feb 2:S1359-

6446(21)00059-3. doi: 10.1016/j.drudis.2021.01.027.

12. Lucianò AM, Perciballi E, Fiore M, Del Bufalo D and Ada Maria Tata. The Combination of the M2

Muscarinic Receptor Agonist and Chemotherapy Affects Drug Resistance in Neuroblastoma Cells. Int. J. Mol. Sci. 2020, 21, 8433; doi:10.3390/ijms21228433.

13. Di Martile M, Garzoli S, Ragno R, Del Bufalo D. Essential Oils and Their Main Chemical Components: The Past 20 Years of Preclinical Studies in Melanoma. Cancers (Basel). 2020 Sep 16;12(9):E2650. doi:

10.3390/cancers12092650.PMID: 32948083.

14. Di Martile M, Gabellini C, Desideri M, Matraxia M, Farini V, Valentini E, Carradori S, Ercolani C, Buglioni S, Secci D, Andreazzoli M, Del Bufalo D, Trisciuoglio D. Inhibition of lysine acetyltransferases impairs tumor angiogenesis acting on both endothelial and tumor cells. J Exp Clin Cancer Res. 2020 Jun

5;39(1):103. doi: 10.1186/s13046-020-01604-z.

15. D’Aguanno S and Del Bufalo D. Inhibition of Anti-Apoptotic Bcl-2 Proteins in Preclinical and Clinical

Studies: Current Overview in Cancer Cells 2020, 9, 1287; doi:10.3390/cells9051287.

16. Di Martile M, Farini V, Consonni FM, Trisciuoglio D, Desideri M, Valentini E, D'Aguanno S, Tupone MG, Buglioni S, Ercolani C, Gallo E, Amadio B, Terrenato I, Foddai ML, Sica A, Del Bufalo D. Melanoma-specific bcl-2 Promotes a Protumoral M2-like Phenotype by Tumor-Associated Macrophages. J Immunother Cancer. 2020 Apr;8(1):e000489. doi: 10.1136/jitc-2019-000489.

17. Romanelli A, Stazi G, Fioravanti R, Zwergel C, Di Bello E, Pomella S, Perrone C, Battistelli C, Strippoli

R, Tripodi M, Del Bufalo D, Rota R, Trisciuoglio D, Mai A, Valente S. Design of First-in-Class DualChem Lett. 2020 Mar 19;11(5):977-983. doi:10.1021/acsmedchemlett.0c00014.

18. Zwergel C, Fioravanti R, Stazi G, Sarno F, Battistelli C, Romanelli A, Nebbioso A, Mendes E, Paulo A, Strippoli R, Tripodi M, Pechalrieu D, Arimondo PB, De Luca T, Del Bufalo D, Trisciuoglio D, Altucci L, Valente S, Mai A. Novel Quinoline Compounds Active in Cancer Cells Through Coupled DNA Methyltransferase Inhibition and Degradation. Cancers (Basel). 2020 Feb 14;12(2):447. doi:

10.3390/cancers12020447.

19. Tupone MG, D'Aguanno S, Di Martile M, Valentini E, Desideri M, Trisciuoglio D, Donzelli S, Sacconi A, Buglioni S, Ercolani C, Biagioni A, Fibbi G, Fattore L, Mancini R, Ciliberto G, Blandino G, Del Bufalo D. microRNA-378a-5p is a Novel Positive Regulator of Melanoma Progression. Oncogenesis.

2020 Feb 14;9(2):22. doi: 10.1038/s41389-020-0203-6.

20. Bosco MC, D'Orazi G, Del Bufalo D. Targeting hypoxia in tumor: a new promising therapeutic strategy. J Exp Clin Cancer Res. 2020 Jan 10;39(1):8. doi: 10.1186/s13046-019-1517-0.

21. Mottini C, Tomihara H, Carrella D, Lamolinara A, Iezzi M, Huang JK, Amoreo CA, Buglioni S, Manni I,

Robinson FS, Minelli R, Kang Y, Fleming JB, Kim MP, Bristow CA, Trisciuoglio D, Iuliano A, Del Bufalo D, Di Bernardo D, Melisi D, Draetta GF, Ciliberto G, Carugo A, Cardone L. Predictive Signatures Inform the Effective Repurposing of Decitabine to Treat KRAS-Dependent Pancreatic Ductal Adenocarcinoma. Cancer Res. 2019 Nov 1;79(21):5612-5625. doi: 10.1158/0008-5472.CAN-19-0187.

22. Trisciuoglio D, Di Martile M, Del Bufalo D. Emerging Role of Histone Acetyltransferase in Stem Cells and Cancer. Stem Cells Int. 2018 Dec 16;2018:8908751. doi: 10.1155/2018/8908751.

23. D’Aguanno S, Valentini E, Tupone MG, Desideri M, Di Martile M, Spagnuolo M, Buglioni S, Ercolani

C, Falcone I, De Dominici M, Milella M, Rizzo MG, Calabretta B, Cota C, Anichini A, Trisciuoglio D, Del Bufalo D. Semaphorin 5A drives melanoma progression: role of Bcl-2, miR-204 and c-Myb. J Exp Clin Cancer Res. 2018 Nov 19;37(1):278. doi: 10.1186/s13046-018-0933-x.

24. Iachettini S, Trisciuoglio D, Rotili D, Lucidi A, Salvati E, Zizza P, Di Leo L, Del Bufalo D, Ciriolo MR, Leonetti C, Steegborn C, Mai A, Rizzo A, and Biroccio A. Pharmacological activation of SIRT6 triggers lethal autophagy in human cancer cells. Cell Death Dis. 2018 Sep 24;9(10):996. doi: 10.1038/s41419-

018-1065-0.

25. Del Curatolo A, Conciatori F, Cesta Incani U, Bazzichetto C, Falcone I, Corbo V, D’Agosto S, Eramo A, Sette G, Sperduti I, De Luca T, Marabese M, Shirasawa S, De Maria R, Scarpa A, Broggini M, Del Bufalo D, Cognetti F, Milella M and Ciuffreda L. Therapeutic potential of combined BRAF/MEK blockade in 1 BRAF-wild type preclinical tumor models. J Exp Clin Cancer Res. 2018 Jul 9;37(1):140. doi: 10.1186/s13046-018-0820-5.

26. Loria R, Laquitana V, Bon G, Trisciuoglio D, Covello R, Amoreo CA, Ferraresi V, Zoccali C, Novello

M, Del Bufalo D, Milella M, D'Incalci M, Biagini R, Falcioni R. HMGA1/E2F1 axis and NFkB pathways regulate liposarcoma progression and trabectedin resistance. Oncogene. 2018 Jul 6. doi: 10.1038/s41388-

018-0394-x.

27. Di Martile M, Desideri M, Tupone MG, Buglioni S, Antoniani B, Mastroiorio C, Falcioni R, Ferraresi V, Baldini N, Biagini R, Milella M, Trisciuoglio D, Del Bufalo D. Histone deacetylase inhibitor ITF2357 leads to apoptosis and enhances doxorubicin cytotoxicity in preclinical models of human sarcoma. Oncogenesis. 2018 Feb 23;7(2):20. doi: 10.1038/s41389-018-0026-x.

28. Monaco G, La Rovere R, Karamanou S, Welkenhuyzen K, Ivanova H, Vandermarliere E, Di Martile M, Del Bufalo D, De Smedt H, Parys JB, Economou A and Bultynck G. A double point mutation at residues Ile14 and Val15 of Bcl-2 uncovers a role for BH4 domain in both protein stability and function. FEBS J.

2018 Jan;285(1):127-145. doi: 10.1111/febs.14324. Epub 2017 Dec 2. PMID: 29131545.

29. Ferrara M, Sessa G, Fiore M, Bernard F, Asteriti IA, Cundari E, Colotti G, Ferla S, Desideri M, Buglioni S, Trisciuoglio D, Del Bufalo D, and Brancale A, Degrassi F. Small molecules targeted to the microtubule-Hec1 interaction inhibit cancer cell growth through microtubule stabilization. Oncogene.

2018 Jan 11;37(2):231-240. doi: 10.1038/onc.2017.320. Epub 2017 Sep 18. PMID: 28925395.

30. Gabellini C, Gómez-Abenza E, Ibáñez-Molero S, Tupone MG, Pérez-Oliva AB, de Oliveira S, Del Bufalo D, Mulero V. Interleukin 8 mediates bcl-xL-induced enhancement of human melanoma cell dissemination and angiogenesis in a zebrafish xenograft model. Int J Cancer. 2018 Feb 1;142(3):584-596. doi: 10.1002/ijc.31075. Epub 2017 Oct 12. PMID: 28949016.

31. Trisciuoglio D, Tupone MG, Desideri M, Di Martile M, Gabellini C, Buglioni S, Pallocca M, Alessandrini G, D’Aguanno S, Del Bufalo D. BCL-XL overexpression promotes tumor progression- associated properties. Cell Death Dis. 2017 Dec 13;8(12):3216. doi: 10.1038/s41419-017-0055-y.

Benedetto A, Mottolese M, Gentile A, Centonze D, Ferrè F, Barilà D. Caspase-8 contributes to angiogenesis and chemotherapy resistance in glioblastoma. Elife. 2017 Jun 8;6. pii: e22593. doi:

10.7554/eLife.22593.

33. Gabellini C, Trisciuoglio D, Del Bufalo D. Non-canonical Role of Bcl-2 and Bcl-xL proteins: relevance of BH4 domain. Carcinogenesis, 2017 Vol. 38, No. 6, 579–587. doi:10.1093/carcin/bgx016.

34. Noto A, De Vitis C, Pisanu ME, Roscilli G, Ricci G, Catizone A, Sorrentino G, Chianese G, Taglialatela-

Scafati O, Trisciuoglio D, Del Bufalo D, Di Martile M, Di Napoli A, Ruco L, Costantini S, Jakopin Z, Budillon A, Melino G, Del Sal G, Ciliberto G, and Mancini R. Stearoyl-CoA-Desaturase (SCD1) regulates lung cancer stemness via stabilization and nuclear localization of YAP/TAZ. Oncogene. 2017

Aug 10;36(32):4671-4672. doi: 10.1038/onc.2017.212. Epub 2017 Jun 19. PMID: 28628115.

35. Kumar Palanisamy S, Trisciuoglio D, Zwergel C, Del Bufalo D, Mai A Metabolite profiling of ascidian.

Styela plicata using LC-MS with multivariate statistical analysis and their Anti-tumor activity. J Enzyme

Inhib Med Chem. 2017 32(1):614-623. doi: 10.1080/14756366.2016.1266344.

36. Milella M, Falcone I, Conciatori F, Matteoni S, Sacconi S, De Luca T, Bazzichetto C, Corbo V, Simbolo M, Sperduti I, Benfante A, Del Curatolo A, Cesta Incani U, Malusa F, Eramo A, Sette G, Scarpa S, Konopleva M, Andreeff M, McCubrey J, Blandino G, Todaro M, Stassi G, De Maria R, Cognetti F, Del Bufalo D, and Ciuffreda L. PTEN status is a crucial determinant of the functional outcome of combined MEK and mTOR inhibition in cancer. Sci Rep. 2017 Feb 21;7:43013. doi: 10.1038/srep43013.