Identifying Potential Drug Targets In Melanoma.

A review submitted to the

Bioinformatics Centre,

Savitribai Phule Pune University, Pune-07

For the degree of

M. Sc. in Bioinformatics

By

Ms.Nayanika Sekhar Das

March 2019

CERTIFICATE

This is to certify that the review entitled **"Identifying Potential Drug Targets In Melanoma",** submitted by **Ms.Das Nayanika** in partial fulfillment of the requirements for the degree of Master of Science in Bioinformatics, has been written satisfactorily by her/him at the Bioinformatics Centre, Savitribai Phule Pune University, Pune-07.

Date: 05/03/2019
Place: Pune

Prof. Sangeeta Sawant
Director
Bioinformatics Centre
Savitribai Phule Pune University, Pune-411007

DECLARATION & UNDERTAKING

I hereby declare that the review entitled, "**Identifying Potential Drug Targets In Melanoma**", submitted in partial fulfillment of the requirements of the degree of Master of Science in Bioinformatics, has been written by me at Bioinformatics Centre, Savitribai Phule Pune University, Pune-07. I further declare that this review or any part there of has not been previously submitted for any degree or diploma of any University.

I also declare that to the best of my ability, I have ensured that the submission made herein, including the main text, table and figures, does not contain any plagiarized material, content or ideas, and that all necessary attributions have been appropriately made and all copyright permissions obtained/cited and acknowledged.

I also declare that any further modification, publication or any other use of this review (either in full or in part), if any, shall be undertaken with prior written consent from the Director, Bioinformatics Centre, Savitribai Phule Pune University, pune-07 and the Review Supervisor/s.

I further state that I shall explicitly mention, "Bioinformatics Centre, Savitribai Phule Pune University" as "place of work" and acknowledge "the M.Sc. Bioinformatics training programme at Savitribai Phule Pune University, Pune-07 for infrastructure and facilities" in the publication (print and online)/of the review article (if any). I shall also acknowledge source of M.Sc. studentship (DBT or IGIB-GNR), if availed.

Date: 05/03/2019 Ms.Nayanika Sekhar Das

Place: Pune BIM_2017_07

ABSTRACT

Melanoma is a form of skin cancer which is non curable.The major types are Superficial spreading melanoma, Lentigo maligna, Acral lentiginous melanoma,Nodular melanoma and Amelanotic melanoma.Moles are representation of any type of melanoma.Stages define the affect of oncogenes.At Stage zero it does not tend to spread very quickly.Stage one spread to the epidermis of the skin.Stage two have a higher risk of extending it's surface to the lymph nodes and further.Stage three and four are advanced types of melanoma which can affect other parts of the body including eyes too.The pathways that are aimed in order to find potential drugs against Melanoma are The MAPK and PI3K-Akt The most aggressive genes also called as oncogenes are BRAF,NRAS,CDK4 and MITF.Therapeutic strategies against BRAF(B-Raf proto-oncogene) a threonine-protein kinase have developed in the recent years and have shown about 30-40% survival rate in patients for almost 6 to 8 months.CDK4 is a cyclin dependent kinase 4 that restricts the normal functioning of cell cycle at a point and progresses for melanoma escalation.MITF is a transcription factor associated oncogene that regulates the cell proliferation,survival of melanoma cells.It regulates the expression of tyrosinase in advanced type of melanoma.NRAS the Neuroblastoma RAS viral oncogene a GTPase protein is the second most important oncogene after BRAF.There are no therapeutic startegies developed for mutant NRAS till date.There are certain MEK inhibitors which are built to cease the functioning of NRAS but they failed to show sufficient affect.Hence,it is highly important to take vital steps towards the development of drugs against different biomarkers of melanoma.This review mainly focuses on what are the drugs available for melanoma and what can be future walk for it.

INTRODUCTION

Melanoma is a form of skin cancer which has no cure till date.It is more widely prone to western parts of the world.It spreads superficially,Later to the internal parts of the skin and other parts of the body in advanced stages.

Symptoms

No symmetricity in shape,rough edges,dark coloured,changes it's size and shape.(https://www.cancercenter.com/cancer-types/melanoma/symptoms)

The other ways of detecting melanoma are :

No healing of sores or moles,redness,pigments,itchiness,pain,blurred vision.(https://www.cancercenter.com/cancer-types/melanoma/symptoms)

Types Of Melanoma

1. Superficial spreading melanoma :

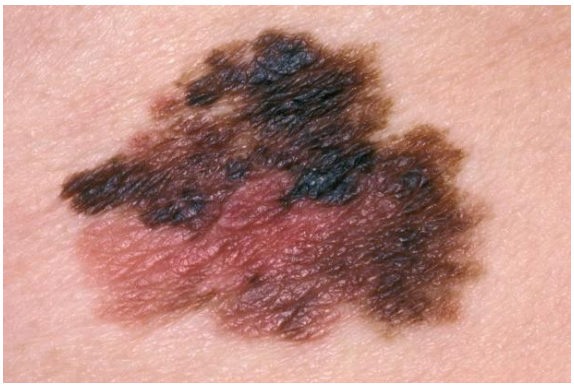

Fig 1.Image has been borrowed from https://www.nhs.uk/conditions/melanoma-skin-cancer/

Darker skins do not show this type of melanoma on a high scale like white or pale skins do.It is only spread over the skin and have an uneven border.It is found only about 10% of the patients affected. (https://www.nhs.uk/conditions/melanoma-skin-cancer/)

2.Nodular Melanoma :

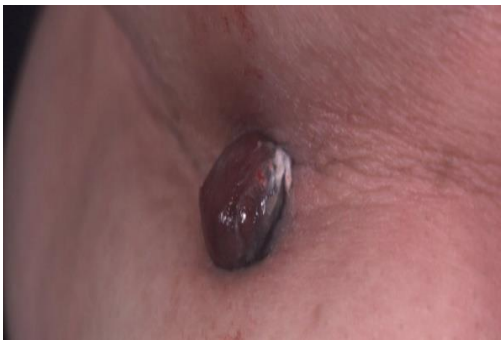

Fig 2.Image has been borrowed from https://www.nhs.uk/conditions/melanoma-skin-cancer/

Nodular type of melanoma grow faster than any other type.Found in the nodes of the skin deep rooted.They cause a lot of pain and this type is a bleeding type of melanoma scar. (https://www.nhs.uk/conditions/melanoma-skin-cancer/)

3. Lentigo maligna melanoma :

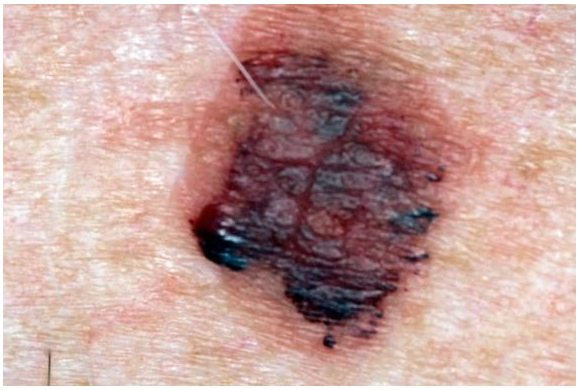

Fig 3.Image has been borrowed from https://www.nhs.uk/conditions/melanoma-skin-cancer/

This type of melanoma is found in people with age more than 60 and above.The areas of skin that are visible to sun are mostly affected by lentigo type of melanogenesis.These prefer to go in the interior of the skin with darker coloured moles. (https://www.nhs.uk/conditions/melanoma-skin-cancer/)

4. Acral lentiginous melanoma :

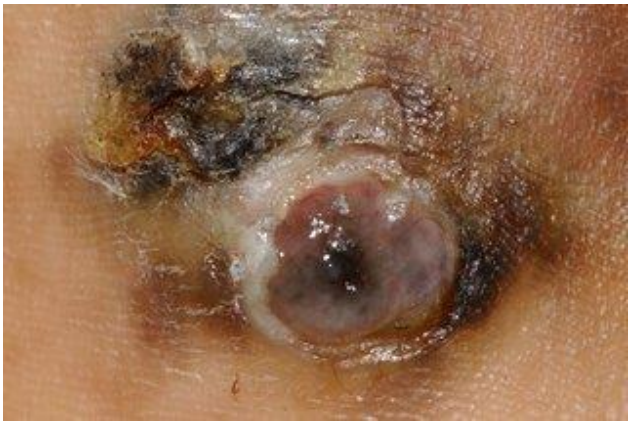

Fig 4.Image has been borrowed from https://www.nhs.uk/conditions/melanoma-skin-cancer/

Acral type of melanoma occur on the nails,closed regions like palms,soles of foot.These are not that often see only in white skin but can be found in any type of skin complexion. (https://www.nhs.uk/conditions/melanoma-skin-cancer/)

5. Amelanotic melanoma :

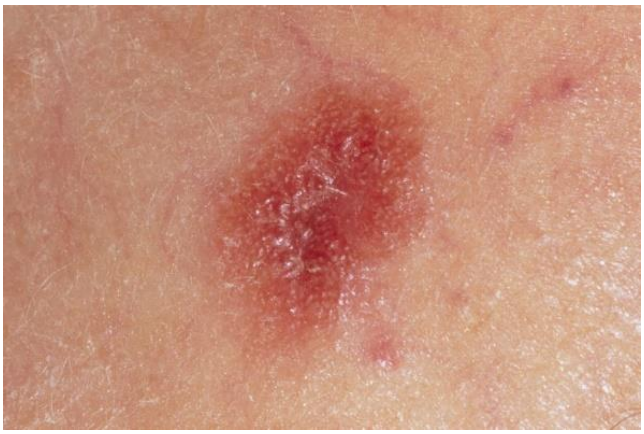

Fig 5.Image has been borrowed from https://www.nhs.uk/conditions/melanoma-skin-cancer/

Amelanotic melanomas do not occur frequently in humans.It is only 5 in 100 type.No marked edges and red in colour. (https://www.nhs.uk/conditions/melanoma-skin-cancer/)

Important Pathways Involved In Melanoma

Table I. The contents of this table has been borrowed from Shtivelman E, Davies MQ, Hwu P, et al. Pathways and therapeutic targets in melanoma. *Oncotarget*. 2014;5(7):1701-52.

PATHWAY	COMPONENTS MUTATED/ACTIVATED	TYPE OF ALTERATION
RAS/RAF/MEK/ERK	NRAS BRAF MEK1	Mutation Mutation Mutation
RAS/PI3K/PTEN/AKT/mTOR	PIK3CA PTEN AKT1,AKT2 ATK3	Mutation Mutation Rare mutation Amplification
CDK	CDK4 CCND1	Mutation/amplification Amplification
MITF	MITF	Mutation/amplification

Melanoma Pathway In Homo Sapiens

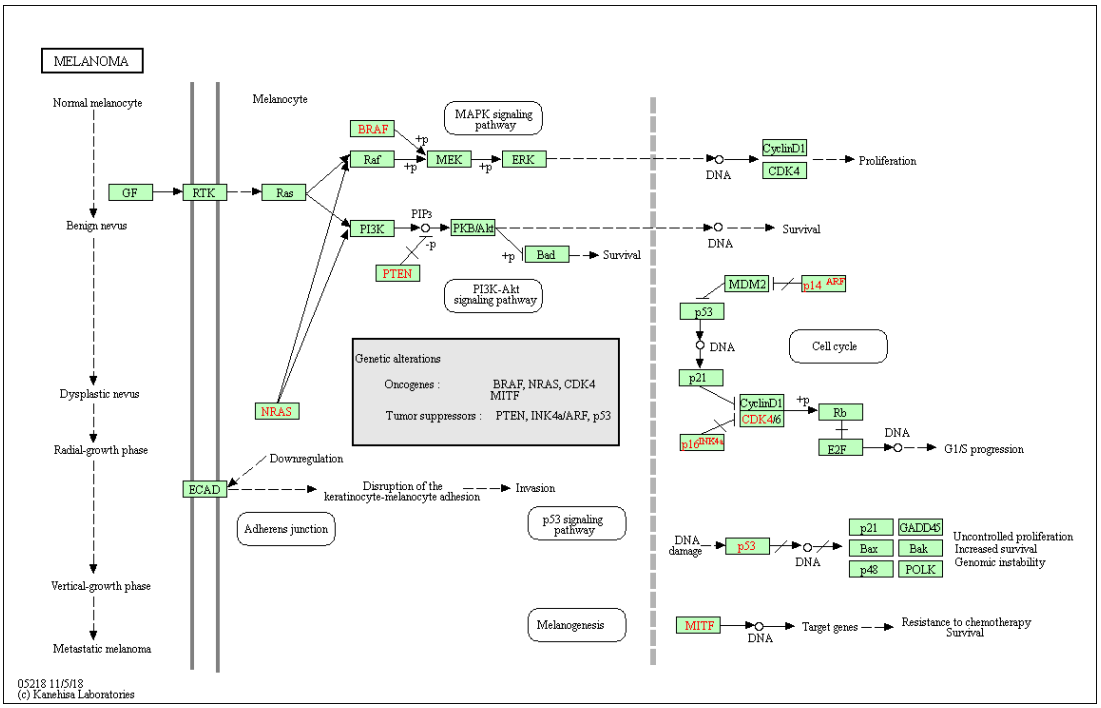

Fig 6.This image has been borrowed from https://www.genome.jp/kegg-bin/show_pathway?hsa05218

MAPK PATHWAY :

MAPK pathway is a mitogen activated kinase pathway(AHMAD NAJEM1,et.al).It is also called as RAS-RAF-MEK-ERK pathway.The activation of this pathway can lead to cell growth and signalling of melanoma cells.This is the most targeted pathway and occurs in the early stages of melanoma.The BRAF in melanoma is the essential target therapy with MEK inhibitors,while the next target that is found is NRAS.NRAS has no particular drug associated with it and hence,finding out potential drugs with the same is the need of the hour.Most melanomas show NRAS mutation and are said to be highly aggressive.Cross talks of pathways are an important way to solve MAPK targeted therapies.By inhibition of PI3K-Akt pathway and not inhibiting V600EB-RAF we can somewhat lead to a better finding towards tumor suppression (Inamdar GS).

PI3K/AKT/mTOR pathway :

PI3K-Akt pathway has a role in RAF and MEK inhibitor combination resistance for a good mixture target of drugs (Siroy A.E., Davies M.A., Lazar A.J.).mTOR mutation yet less common than RAF or RAS is considered to be an important pathway to abolish meloanocytic cells.The mutations found in mTOR are in acral,mucosal form(Yan Kong,et.al).PTEN(phosphatase and tensin) acts as a tumor suppressing unit for this pathway.The intricate nature of this pathway has made it difficult to develop clinical activity or immune based therapy.

CDK4 Pathway :

Disfunctioning of CDK4 cycle is commonly present in melanoma and therefore becomes and essential target.This pathway follows the cell cycle arrest and death of healthy cells leading to progression of melanocytes (Sheppard KE1, McArthur GA).The cell check points are present where each CDKs handle them at various locations.The dormant cells enter into G1 phase from G0 phase.(Lee B, McArthur GA)BRAF has proved to be an essential biomarker while treating melanoma by CDK4 pathway.The connection of complementary MAPK and CDK4 pathway together can act as a specific mutant NRAS inhibitory circuits(Lee B, McArthur GA).

MITF Pathway :

MITF (microphthalmia-associated transcription factor) is a transcription factor found mostly in malignant melanoma(Hartman ML, Czyz M).mRNA act as an unlikely change in MITF transcript(Hartman ML, Czyz M). Many such mRNAs and siRNAs promote downregulation of protein synthesis (Hartman ML, Czyz M).The activity is shown on the post translational modification and found in the advanced III and IV stages of melanoma (Hartman ML, Czyz M).

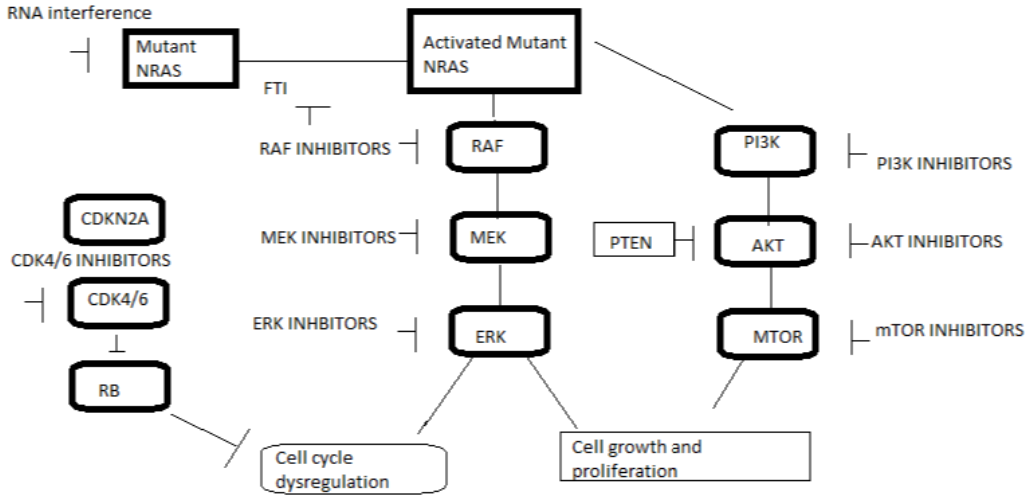

Fig 7. Possible targeted therapies.Contents have been obtained from (Douglas B. Johnson, M.D. and Igor Puzanov, M.D. Department of Medicine, Division of Hematology/Oncology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 777 Preston Research Building, 2220 Pierce Avenue, Nashville, TN 37232, USA. Treatment of NRAS-Mutant Melanoma. Curr Treat Options Oncol. 2015 April ; 16(4): 15. doi:10.1007/s11864-015-0330-z.).Drawn in paint.

Clinically Approved Drugs Against Melanoma :

1. Trametinib :

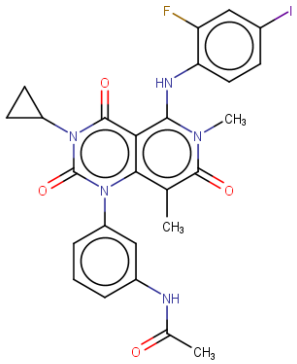

Fig 8.Structure has been drawn using Marvin Sketch.Smiles have been obtained from ZINC15 database.

Trametinib was basically designed for BRAF.But a few patients who had mutant NRAS affected cells were tested with this drug.It has FDA approval for consumption.It has be proposed that a mixture of Trametinib and other drugs might play a vital role in NRAS inhibition in future(Douglas B. Johnson, M.D. and Igor Puzanov).

2.Binimetinib (MEK162) :

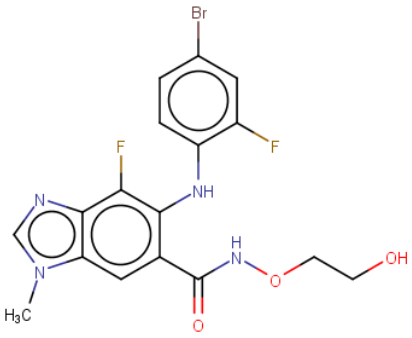

Fig 9.Structure has been drawn using Marvin Sketch. Smiles have been obtained from ZINC15 database.

Patients with solid tumors were targeted using Binimetinib.It is a type of MEK inhibitor specifically for mutant NRAS(AHMAD NAJEM1,et.al).It showed considerably good amount of recovery to mutant NRAS activity than any other inhibitors till today.

3. RO4987655 :

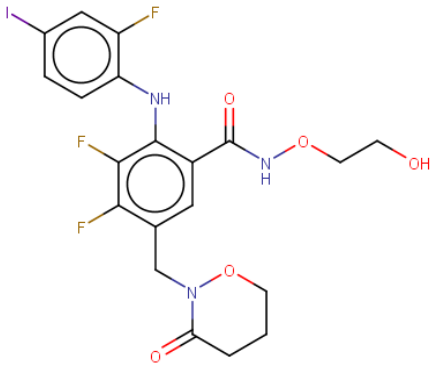

Fig 10.Structure has been drawn using Marvin Sketch. Smiles have been obtained from ZINC15 database.

RO4987655,a MEK inhibitor is undergoing a clinical trial.Till today it shows only 13% recovery in patients (Douglas B. Johnson, M.D. and Igor Puzanov).

4. Farnesyltransferase inhibitors (FTIs) :

A cysteine residue of post translational modification of RAS are the Farnesyltransferase inhibitors.Initially they were developed for targeting NRAS in combination to other drugs.But since no potential output was obtained,clinical trails have stopped taking place now (Douglas B. Johnson, M.D. and Igor Puzanov).

5.Cobimetinib :

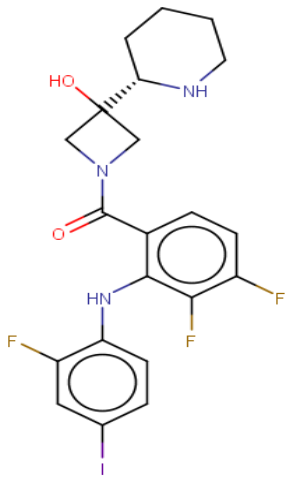

Fig 11.Structure has been drawn using Marvin Sketch. Smiles have been obtained from ZINC15 database.

Cobimetinib shows a great yield of activity when combined with Vemurafenib.About more than 80% reactivity of BRAF 600-mutant was observed with this therapy (Douglas B. Johnson, M.D. and Igor Puzanov).

6. Vemurafenib :

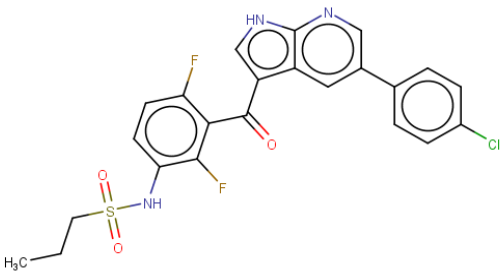

Fig 12.Structure has been drawn using Marvin Sketch. Smiles have been obtained from ZINC15 database.

It is a BRAF drug .BRAF-600 have resulted into a good response to this drug and now is widely used one to inhibit this marker(Douglas B. Johnson, M.D. and Igor Puzanov).

7.Selumetinib :

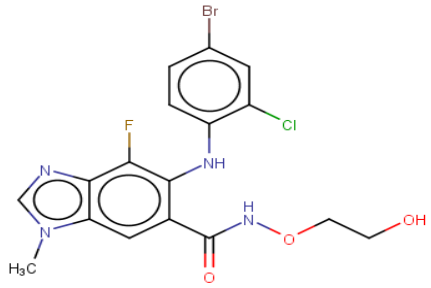

Fig 13.Structure has been drawn using Marvin Sketch. Smiles have been obtained from ZINC15 database.

A MEK associated inhibitor.In humans it shows a half way response to NRAS activity while in xenographs it is a therapy for RAS and RAF (Douglas B. Johnson, M.D. and Igor Puzanov).

8.Dabrafenib :

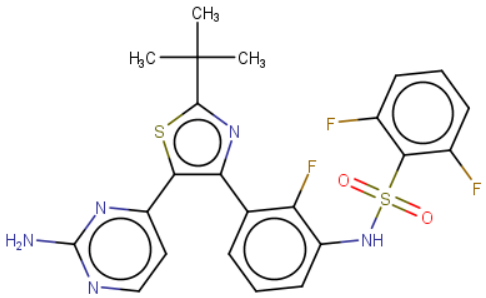

Fig 14. Structure has been drawn using Marvin Sketch. Smiles have been obtained from ZINC15 database.

Presently under clinical trails.Dabrafenib was developed for treatment against BRAF.(. Douglas B. Johnson, M.D. and Igor Puzanov)

CONCLUSION

Among the three important pathways CDK4,PI3k-Akt and MAPK,the CDK4 pathway has not been directly targeted till today.The CDK4 cell cycle pathway in melanoma can be selected as the aim of supressing melanoma.MEK inhibitors however have promised to be showing considerably higher effect in future which are under clinical trials today.Also a combination of MEK RAF and ERK inhibitors can be made to find proper drugs exactly at mutant positions in NRAS.There are no vaccines developed for NRAS-BRAF till the present time.An able peptide which can be called a vaccine on the NRAS-BRAF surface can also prove to deprive melanoma to some extent at a very early stage.Glycoprotein Non-Metastatic Melanoma Protein B is a transmembrane protein found in all the cells of melanoma whether mutated or wild type.This GPNMB protein B enables cell ceel response as it acts as a surface linking cell cell protein.It has an ectodomain (RGD)-Arginine,Glycine and Aspartic acid which is shed off,if this domain is refrained from shedding off,retains in the surface itself,malignant growth and metastasis can be stopped in future.Also as in prostate cancer silencing of GPNMB was performed,the same approach can be done for melanocytic cells.Which might make a difference towards looking at several ways to inhibit the growth of melanoma cells in future.

References

1.Shtivelman E, Davies MQ, Hwu P, et al. Pathways and therapeutic targets in melanoma. *Oncotarget*. 2014;5(7):1701-52.

2. AHMAD NAJEM1*, MOHAMMAD KRAYEM1*, ANNE PERDRIX1,2, JOSEPH KERGER3, AHMAD AWADA3, FABRICE JOURNE1,4 and GHANEM GHANEM. New Drug Combination Strategies in Melanoma: Current Status and Future Directions. ANTICANCER RESEARCH 37: 5941-5953 (2017) doi:10.21873/anticanres.12041

3. Amaral T1, Sinnberg T2, Meier F3, Krepler C4, Levesque M5, Niessner H2, Garbe C2. The mitogen-activated protein kinase pathway in melanoma part I - Activation and primary resistance mechanisms to BRAF inhibition. Eur J Cancer. 2017 Mar;73:85-92.

4. Inamdar GS, Madhunapantula SV, Robertson GP. Targeting the MAPK pathway in melanoma: why some approaches succeed and other fail. Biochem Pharmacol. 2010;80(5):624-37.

5. Yan Kong, Lu Si, Yiqian Li, Xiaowen Wu, Xiaowei Xu, Jie Dai, Huan Tang, Meng Ma, Zhihong Chi, Xinan Sheng, Chuanliang Cui and Jun Guo.Analysis of mTOR Gene Aberrations in Melanoma Patients and Evaluation of Their Sensitivity to PI3K–AKT–mTOR Pathway Inhibitors.Clin Cancer Res February 15 2016 (22) (4) 1018-1027; DOI: 10.1158/1078-0432.CCR-15-1110

6. Siroy A.E., Davies M.A., Lazar A.J. (2016) The PI3K-AKT Pathway in Melanoma. In: Torres-Cabala C., Curry J. (eds) Genetics of Melanoma. Cancer Genetics. Springer, New York, NY

7. Sheppard KE1, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma.Clin Cancer Res. 2013 Oct 1;19(19):5320-8. doi: 10.1158/1078-0432.CCR-13-0259.

8. Lee B, McArthur GA. CDK4 inhibitors an emerging strategy for the treatment of melanoma. Melanoma Manag. 2015;2(3):255-266.

9.Hartman ML, Czyz M. MITF in melanoma: mechanisms behind its expression and activity. *Cell Mol Life Sci*. 2014;72(7):1249-60.

10. Douglas B. Johnson, M.D. and Igor Puzanov, M.D. Department of Medicine, Division of Hematology/Oncology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 777 Preston Research Building, 2220 Pierce Avenue, Nashville, TN 37232, USA. Treatment of NRAS-Mutant Melanoma. Curr Treat Options Oncol. 2015 April ; 16(4): 15. doi:10.1007/s11864-015-0330-z.

URLs

1. https://www.cancercenter.com/cancer-types/melanoma/symptoms
2. https://www.genome.jp/kegg-bin/show_pathway?hsa05218
3. https://www.nhs.uk/conditions/melanoma-skin-cancer/
4. https://www.uniprot.org/uniprot/P01111#sequences