HYPOTHESIS AND THEORY

Possible effects of sirolimus treatment on the long-term efficacy of COVID-19 vaccination in patients with β-thalassemia: A theoretical perspective

MATTEO ZURLO[1], FRANCESCO NICOLI[2], MONICA BORGATTI[1], ALESSIA FINOTTI[1] and ROBERTO GAMBARI[1]

Departments of [1]Life Sciences and Biotechnology, and [2]Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, I-44121 Ferrara, Italy

Received October 25, 2021; Accepted January 3, 2022

DOI: 10.3892/ijmm.2022.5088

Abstract. The pandemic caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2), responsible for coronavirus disease 2019 (COVID-19) has posed a major challenge for global health. In order to successfully combat SARS-CoV-2, the development of effective COVID-19 vaccines is crucial. In this context, recent studies have highlighted a high COVID-19 mortality rate in patients affected by β-thalassemia, probably due to their co-existent immune deficiencies. In addition to a role in the severity of SARS-CoV-2 infection and in the mortality rate of COVID-19-infected patients with thalassemia, immunosuppression is expected to deeply affect the effectivity of anti-COVID-19 vaccines. In the context of the interplay between thalassemia-associated immunosuppression and the effectiveness of COVID-19 vaccines, the employment of immunomodulatory molecules is hypothesized. For instance, short-term treatment with mammalian target of rapamycin inhibitors (such as everolimus and sirolimus) has been found to improve responses to influenza vaccination in adults, with benefits possibly persisting for a year following treatment. Recently, sirolimus has been considered for the therapy of hemoglobinopathies (including β-thalassemia). Sirolimus induces the expression of fetal hemoglobin (and this may contribute to the amelioration of the clinical parameters of patients with β-thalassemia) and induces autophagy (thereby reducing the excessive levels of α-globin). It may also finally contribute to the mobilization of erythroid cells from the bone marrow (thereby reducing anemia). In the present study, the authors present the hypothesis that sirolimus treatment, in addition to its beneficial effects on erythroid-related parameters, may play a crucial role in sustaining the effects of COVID-19 vaccination in patients with β-thalassemia. This hypothesis is based on several publications demonstrating the effects of sirolimus treatment on the immune system.

Correspondence to: Professor Roberto Gambari, Department of Life Sciences and Biotechnology, University of Ferrara, Via Fossato di Mortara 74, I-44121 Ferrara, Italy
E-mail: gam@unife.it

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; mTOR, mammalian target of rapamycin; ARDS, acute respiratory distress syndrome; IHD, ischemic heart disease; COPD, chronic obstructive pulmonary disease; NK cells, natural killer cells; DC, dendritic cell; PBMC, peripheral blood mononuclear cell; HLA, human leukocyte antigen; APC, antigen-presenting cell

Key words: COVID-19, vaccines, sirolimus, β-thalassemia, mTOR

Introduction

The dramatic pandemic caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2), responsible for coronavirus disease 2019 (COVID-19) has posed a major new challenge for human health worldwide (1,2). The rapidly increasing amounts of research on COVID-19 has allowed for the understanding of several aspects of the pathophysiology of SARS-CoV-2, including the key steps of infection, the hyper-inflammatory state (termed 'cytokine storm') leading to acute respiratory distress syndrome (ARDS), and the fact that severe forms of this disease are more frequently observed in elderly patients, particularly when associated with underlying comorbidities, such as hypertension, diabetes, obesity, ischemic heart disease (IHD) and chronic obstructive pulmonary disease (COPD) (2,3). Moreover, the mortality rate caused by COVID-19 has been found to increase exponentially with age, even considering the very high variability in the reported mortality rates by studies employed on very different testing strategies and therapeutic interventions (4-6).

Recent research has highlighted a high COVID-19 mortality rate in patients with β-thalassemia (7), probably due to co-existent immune deficiencies (8). Immune dysfunctions characterizing patients with thalassemia include changes in lymphocyte subsets, such as the accumulation of suppressor T-cells and the reduced proliferative capacity and numbers of T-helper cells, as well as the defective activity of natural killer (NK) cells. Similarly, an altered humoral immunity has been found in patients with β-thalassemia (9). Subjects presenting

with similar immune system defects (i.e., the elderly) exhibit a marked susceptibility to severe COVID-19-related symptoms (4-6,10). This suggests that β-thalassemia-associated immunosuppression should be actively targeted to protect these patients.

In addition to a role in the severity of SARS-CoV-2 infection and in the mortality of the COVID-19-infected patients with thalassemia, immunosuppression is expected to greatly affect the effectiveness of anti-COVID-19 vaccines. This is a key issue, since it is widely accepted that the development of effective COVID-19 vaccination is crucial in order to successfully combat SARS-CoV-2 (11-13). In this context, the need for prospective immunosurveillance studies in order to estimate the duration of immunity is of utmost importance and impact (11-14). Effective and long-lasting COVID-19 vaccination will require interventions that generate potent humoral and cellular responses against SARS-CoV-2 antigens (12,13). In this respect, one of the unanswered issues regarding COVID-19 vaccination is the length of time this approach will protect the vaccinated population from infection by SARS-CoV-2 and from the development of severe COVID-19-associated symptoms (12,13).

In the context of the interplay between thalassemia-associated immunosuppression and the effectiveness of COVID-19 vaccines, the employment of immunomodulatory molecules has been considered. For instance, short-term treatment with mammalian target of rapamycin (mTOR) inhibitors (such as everolimus and sirolimus) has been found to improve responses to influenza vaccination in adults, with benefits possibly persisting for a year following treatment (15,16). Such drugs suppress excess inflammation, while also improving innate immunity.

Sirolimus has been considered for the therapy of hemoglobinopathies (for example β-thalassemia and sickle-cell disease) (17-22). Two clinical trials based on the employment of sirolimus for thalassemia have been activated, NCT03877809 (A Personalized Medicine Approach for β-thalassemia Transfusion Dependent Patients: Testing Sirolimus in a First Pilot Clinical Trial) and NCT04247750 (Treatment of β-thalassemia Patients with Rapamycin: From Pre-clinical Research to a Clinical Trial), both using low doses of sirolimus for a 12-month period. The rationale of these two trials is that sirolimus may be of interest for use in β-thalassemia, since it induces the expression of fetal hemoglobin (and this may contribute to ameliorate the clinical parameters of these patients), induces autophagy (thereby reducing the excess of α-globin) and, finally, may contribute to mobilization of erythroid cell from the bone marrow (thereby reducing anemia). In addition to these positive effects on the hematopoietic system, sirolimus may improve the immune system of these patients. This may be a crucial issue, particularly considering that the majority of patients with β-thalassemia who are currently being vaccinated against COVID-19 are in the 45-60 age category. Moreover, it should be underlined that sirolimus and sirolimus analogs (such as everolimus) are extensively used in routine therapy and in clinical studies for the treatment of other diseases, such as renal, cardiac and liver transplantation (23-26), systemic lupus erythematosus (27), lymphangioleiomyomatosis (28), tuberous sclerosis complex (29), recurrent meningioma (30), pancreatic neuroendocrine tumors (31), advanced differentiated thyroid cancers (32), advanced breast cancer (33), diffuse large B-cell lymphomas (34), metastatic renal cell carcinoma (35).

The hypothesis

The working hypothesis is that use of sirolimus can sustain the effectiveness of COVID-19 vaccination in patients with β-thalassemia (Fig. 1) (36). This hypothesis is based on several publications demonstrating the effects of sirolimus on the immune system, as well as on the growing interest for immunomodulators functioning through metabolic manipulation (37). For instance, Amiel *et al* (38) demonstrated that sirolimus promoted dendritic cell (DC) activation and enhanced therapeutic autologous vaccination in mice. These findings define mTOR as a molecular target for augmenting DC survival and activation, and document a novel pharmacologic approach for enhancing the efficacy of therapeutic autologous DC vaccination. In addition, Araki *et al* (39) proposed that sirolimus improved both the quantity and quality of memory $CD8^+$ T-cells induced by viral infection and vaccination, demonstrating that mTOR is also a major regulator of memory $CD8^+$ T-cell differentiation. These discoveries have implications for the development of novel vaccine regimens, and sirolimus can thus have potential for use in improving vaccine efficacy. Notably, the timing of treatment may be of utmost importance. Indeed, mTOR activity is required for B- and T-cell priming, thus arguing against a concomitant use of sirolimus together with vaccination (40,41). Conversely, mTOR inhibition may be crucial for the maintenance of memory lymphocytes (39,42), thus potentially prolonging vaccine immunogenicity. It was thus hypothesized that sirolimus may be tested for possible administration during the early memory phases; i.e., 30-60 days following vaccination.

The information on the possible effects of sirolimus on vaccines is also of great interest considering that sirolimus is extensively used in routine therapy and in clinical studies for other diseases. In addition, the campaign for COVID-19 vaccination is ongoing and will include patients presently being treated with sirolimus.

This hypothesis is sustained by preliminary results obtained in the concluded NCT03877809 trial, indicating that treatment with sirolimus did not lead to a major alteration of the immunophenotype. In particular, the *in vivo* treatment of patients with β-thalassemia with a daily administration of 0.5 mg of sirolimus does not affect the $CD8^+$ T-lymphocyte population over the period of 180 days of therapy. This conclusion was achieved by flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) aimed at assessing the preservation of different immune cell subsets (B-cells, regulatory and conventional $CD4^+$ T-cells, $CD8^+$ T-cells and monocytes) (unpublished data).

Possible experimental evaluation of the hypothesis

In order to verify whether sirolimus treatment affects COVID-19 vaccine immune memory maintenance, two study groups are required, both undergoing COVID-19 vaccination: The first vaccinated cohort can be treated daily with 0.5-2 mg sirolimus for 6 months, that will generate a blood

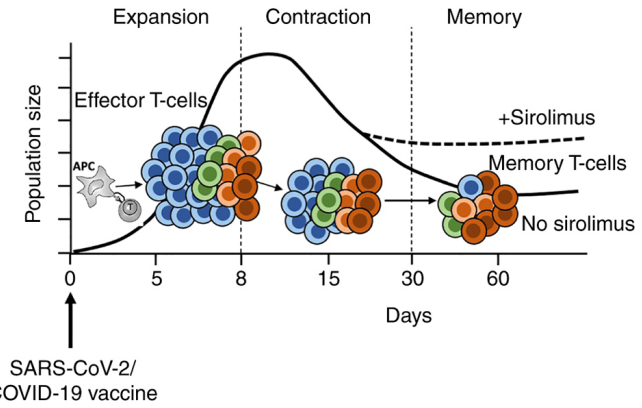

Figure 1. Possible effects of sirolimus on memory T-cells following COVID-19 vaccination. APCs present the vaccine-produced viral antigens to naïve T-cells, initiating a program of clonal expansion (expansion phase) and differentiation into effector T-cells. The functions of these cells include the secretion of pro-inflammatory cytokines (such as TNF-α, IFN-γ and IL-2) and cytotoxic molecules (such as perforin and granzymes). The contraction phase follows vaccine clearance and is characterized by the apoptosis of an important proportion of T-cells. In the memory phase (that can persist for years), memory T-cells remain present in the host and can rapidly expand and acquire effector functions in the case of a secondary exposure to the same vaccine or after infection by the virus against which the vaccine has been developed (36). The hypothesis is that sirolimus treatment after the expansion phase may provide a higher quantity/quality of memory T-cells during the contraction and memory phases. APCs, antigen-presenting cells; COVID-19, coronavirus disease 2019.

concentration ranging between 2-2.5 ng/ml in these patients. The second (control) vaccinated cohort will not be treated with sirolimus. Blood sampling for PBMCs and plasma isolation can be performed in both cohorts, at 1-2 months after the second dose of the vaccine and before commencing treatment with sirolimus; the subsequent samplings will be performed after 90, 180 and 360 days.

Concerning general/non-specific sirolimus-mediated effects on immune-cell subsets, longitudinal immunophenotypic analyses of PBMCs are necessary to assess any eventual fluctuation in the proportion of myeloid and lymphoid cells as well as of specific T- and B-lymphocyte subsets (naive, memory and suppressor cells). This will allow the study of possible modulations of immune cells in the cohort of patients being treated with sirolimus after vaccination compared to those who are only vaccinated, with particular interest in some subpopulations that may be altered in patients with thalassemia and/or be positively modulated by sirolimus (for example NK cells, DCs and memory $CD8^+$ T-lymphocytes).

To assess whether sirolimus improves vaccine-specific memory cellular immunity, the frequency of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ memory T-cells should be measured at different time points. In this respect, several approaches have been envisaged, spanning from the use of tetramers [for defined human leukocyte antigen (HLA)-haplotypes] to the use of pools of overlapping peptides covering different HLA alleles (43,44) or of specific epitopes restricted for specific HLAs (45). As it may be speculated that sirolimus may exert effects on all the memory T-cell compartments, the assessment of recall responses towards other previous encountered antigens could also be performed, e.g., through the use of HLA class I- and II-presented peptide pools containing various antigenic stimuli (Epstein-Barr virus, cytomegalovirus, tetanus and flu). This approach could help to elucidate whether sirolimus limits the normal decline in function that is expected in the passage of time.

To evaluate whether the intake of sirolimus improves vaccine-specific memory humoral immunity, the titers of binding and/or neutralizing specific antibodies against the SARS-CoV-2 spike protein induced by vaccination should be measured longitudinally. This analysis, in addition to verifying the immunomodulatory effect of sirolimus, may provide an estimate of how effective COVID-19 vaccines are over time.

Patients with thalassemia exhibit several immune alterations which may severely affect vaccine effectiveness or in general, the susceptibly to antigenic challenges. Thus, beyond the effects of sirolimus on COVID-19 vaccine responsiveness, its use may be envisaged to restore these immunological abnormalities. For instance, DC maturation is reduced in patients with thalassemia. Therefore, its induction from monocytes in patients before and after treatment could be assessed. In addition, patients with thalassemia present with an increased number and functionality of suppressor T-cells and reduced T-helper cell proliferation. Thus, taking advantage of the proposed research, it can be eventually estimated whether sirolimus can restore the balance and functionality within the T-cell subsets.

Additionally, to ensure the safety profile of sirolimus treatment and since the patient's inflammatory state may influence the vaccine response (46), a qualitative-quantitative analysis of the cytokines involved in adaptive immunity and inflammation in the plasma of patients treated or not with sirolimus may be appropriate.

Conclusions and future perspectives

The expected outcomes of the research activity finalized to examine the effects of *in vivo* treatment with sirolimus in patients with β-thalassemia vaccinated against SARS-CoV-2 are several and crucial. A first point is related to the detection of SARS-CoV-2-specific IgG in the plasma of treated patients and the evaluation of the effects of sirolimus. A second point is the quantification and determinations of the biological activity of SARS-CoV-2-specific memory T-cells in patients with β-thalassemia vaccinated against COVID-19. This point is crucial since molecules potentiating vaccination may be of great interest in a pandemic period in which the administration of a third dose of anti-SARS-CoV-2 vaccines is ongoing in several countries. In this respect, the overall effects of sirolimus treatment on the immune defects of patients with β-thalassemia is a key factor for determining whether this treatment may be proposed.

It should be noted that the impact of this research activity is not limited the sirolimus-treated patients with β-thalassemia. In fact, considering that mTOR inhibitors, such as sirolimus are employed in the treatment of a large variety of pathologies (23-36), the number of patients taking advantages in the case this hypothesis will be confirmed, is relevant. Moreover, it can be envisaged that sirolimus treatment could be used to improve COVID-19 vaccine responses in other populations as well, such as the elderly where similar approaches have already

been shown to improve the efficacy of the flu vaccine (47,48). On the other hand, it should be emphasized that the importance of testing geroprotective drugs (such as sirolimus) will extend far beyond the COVID-19 pandemic to improve overall health resilience of aged populations (46,49,50).

Finally, mTOR inhibitors (such as sirolimus and metformin) are at present undergoing clinical trials as anti-COVID-19 drugs (NCT04461340 and NCT04510194) (51), also considering their effects on activation of autophagy, that is deeply down-regulated during SARS-CoV-2 infection (52). In consideration of the multiple biochemical and cellular effects of sirolimus against SARS-CoV2, it can be considered as a repurposed drug for anti-COVID-19 therapy (53).

In conclusion, the proposed approaches may lead to the development of protocols for sustaining the effects of COVID-19 vaccines in fragile subjects. This is a major issue in the management of patients with COVID-19 in the future and in planning mass immunization strategies finalized in reaching the herd immunity in short period of time.

Acknowledgements

The authors would like to thank Dr Maria Rita Gamberini (Day Hospital Thalassemia, Arcispedale S. Anna, Ferrara) for her support and helpful discussions.

Funding

The present study was supported by the Wellcome Trust (United Kingdom, Innovator Award 208872/Z/17/Z), by AIFA (Agenzia Italiana del Farmaco, Italy, AIFA-2016-02364887) and by the MUR-FISR COVID-miRNAPNA Project (FISR2020IP_04128).

Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

MZ, RG and FN were involved in the conceptualization of the study. AF, MB and RG were involved in the writing and preparation of the original draft, and in the writing, reviewing and editing of the study. MZ and FN were involved in the processing of the figure. RG and AF supervised the study. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, *et al*: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395: 497-506, 2020.
2. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, *et al*: Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 130: 2620-2629, 2020.
3. Mahmudpour M, Roozbeh J, Keshavarz M, Farrokhi S and Nabipour I: COVID-19 cytokine storm: The anger of inflammation. Cytokine 133: 155151, 2020.
4. Nicoli F, Solis-Soto MT, Paudel D, Marconi P, Gavioli R, Appay V and Caputo A: Age-related decline of de novo T cell responsiveness as a cause of COVID-19 severity. Geroscience 42: 1015-1019, 2020.
5. Nikolich-Zugich J, Knox KS, Rios CT, Natt B, Bhattacharya D and Fain MJ: SARS-CoV-2 and COVID-19 in older adults: What we may expect regarding pathogenesis, immune responses, and outcomes. Geroscience 42: 505-514, 2020.
6. Genebat M, Tarancón-Díez L, de Pablo-Bernal R, Calderón A, Muñoz-Fernández MÁ and Leal M: Coronavirus disease (COVID-19): A Perspective from Immunosenescence. Aging Dis 12: 3-6, 2021.
7. Zafari M, Rad MTS, Mohseni F and Nikbakht N: β-Thalassemia Major and Coronavirus-19, mortality and morbidity: A systematic review study. Hemoglobin 45: 1-4, 2021.
8. Farmakis D, Giakoumis A, Polymeropoulos E and Aessopos A: Pathogenic aspects of immune deficiency associated with beta-thalassemia. Med Sci Monit 9: RA19-RA22, 2003.
9. Ghaffari J, Abediankenari S and Nasehi M: Thalassemia and immune system dysfunction-review article. Int J Curr Res 3: 105-108, 2011.
10. Blagosklonny MV: From causes of aging to death from COVID-19. Aging (Albany NY) 12: 10004-10021, 2020.
11. Kaur SP and Gupta V: COVID-19 vaccine: A comprehensive status report. Virus Res 288: 198114, 2020.
12. Chakraborty C, Sharma AR, Bhattacharya M, Sharma G, Saha RP and Lee SS: Ongoing clinical trials of vaccines to fight against COVID-19 pandemic. Immune Netw 21: e5, 2021.
13. Dorrington MG and Bowdish DM: Immunosenescence and novel vaccination strategies for the elderly. Front Immunol 4: 171, 2013.
14. Tsatsakis A, Vakonaki E, Tzatzarakis M, Flamourakis M, Nikolouzakis TK, Poulas K, Papazoglou G, Hatzidaki E, Papanikolaou NC, Drakoulis N, *et al*: Immune response (IgG) following full inoculation with BNT162b2 COVID-19 mRNA among healthcare professionals. Int J Mol Med 48: 200, 2021.
15. Mannick JB, Del Giudice G, Lattanzi M, Valiante NM, Praestgaard J, Huang B, Lonetto MA, Maecker HT, Kovarik J, Carson S, *et al*: mTOR inhibition improves immune function in the elderly. Sci Transl Med 6: 268ra179, 2014.
16. Mannick JB, Morris M, Hockey HP, Roma G, Beibel M, Kulmatycki K, Watkins M, Shavlakadze T, Zhou W, Quinn D, *et al*: TORC1 inhibition enhances immune function and reduces infections in the elderly. Sci Transl Med 10: eaaq1564, 2018.
17. Mischiati C, Sereni A, Lampronti I, Bianchi N, Borgatti M, Prus E, Fibach E and Gambari R: Rapamycin-mediated induction of gamma-globin mRNA accumulation in human erythroid cells. Br J Haematol 126: 612-621, 2004.
18. Fibach E, Bianchi N, Borgatti M, Zuccato C, Finotti A, Lampronti I, Prus E, Mischiati C and Gambari R: Effects of rapamycin on accumulation of alpha-, beta- and gamma-globin mRNAs in erythroid precursor cells from beta-thalassaemia patients. Eur J Haematol 77: 437-441, 2006.
19. Zuccato C, Bianchi N, Borgatti M, Lampronti I, Massei F, Favre C and Gambari R: Everolimus is a potent inducer of erythroid differentiation and gamma-globin gene expression in human erythroid cells. Acta Haematol 117: 168-176. 2007.
20. Pecoraro A, Troia A, Calzolari R, Scazzone C, Rigano P, Martorana A, Sacco M, Maggio A and Di Marzo R: Efficacy of rapamycin as inducer of HbF in primary erythroid cultures from sickle cell disease and beta-thalassemia patients. Hemoglobin 39: 225-229, 2015.
21. Gaudre N, Cougoul P, Bartolucci P, Dörr G, Bura-Riviere A, Kamar N and Del Bello A: Improved fetal hemoglobin with mTOR inhibitor-based immunosuppression in a kidney transplant recipient with sickle cell disease. Am J Transplant 17: 2212-2214, 2017.

22. Al-Khatti AA and Alkhunaizi AM: Additive effect of Sirolimus and hydroxycarbamide on fetal haemoglobin level in kidney transplant patients with sickle cell disease. Br J Haematol 185: 959-961, 2019.
23. Kahan BD: Sirolimus: A new agent for clinical renal transplantation. Transplant Proc 29: 48-50, 1997.
24. Hernández D, Martínez D, Gutiérrez E, López V, Gutiérrez C, García P, Cobelo C, Cabello M, Burgos D, Sola E and González-Molina M: Clinical evidence on the use of anti-mTOR drugs in renal transplantation. Nefrologia 31: 27-34, 2011.
25. Schaffer SA and Ross HJ: Everolimus: Efficacy and safety in cardiac transplantation. Expert Opin Drug Saf 9: 843-854, 2010.
26. Tang CY, Shen A, Wei XF, Li QD, Liu R, Deng HJ, Wu YZ and Wu ZJ: Everolimus in de novo liver transplant recipients: A systematic review. Hepatobiliary Pancreat Dis Int 14: 461-469, 2015.
27. Ji L, Xie W and Zhang Z: Efficacy and safety of Sirolimus in patients with systemic lupus erythematosus: A systematic review and meta-analysis. Semin Arthritis Rheum 50: 1073-1080, 2020.
28. Wang Q, Luo M, Xiang B, Chen S and Ji Y: The efficacy and safety of pharmacological treatments for lymphangioleiomyomatosis. Respir Res 21: 55, 2020.
29. Sasongko TH, Ismail NF and Zabidi-Hussin Z: Rapamycin and rapalogs for tuberous sclerosis complex. Cochrane Database Syst Rev 7: CD011272, 2016.
30. Graillon T, Sanson M, Campello C, Idbaih A, Peyre M, Peyrière H, Basset N, Autran D, Roche C, Kalamarides M, *et al*: Everolimus and octreotide for patients with recurrent meningioma: Results from the phase II CEVOREM trial. Clin Cancer Res 26: 552-557, 2020.
31. Gallo M, Malandrino P, Fanciulli G, Rota F, Faggiano A and Colao A; NIKE Group: Everolimus as first line therapy for pancreatic neuroendocrine tumours: Current knowledge and future perspectives. J Cancer Res Clin Oncol 143: 1209-1224, 2017.
32. Manohar PM, Beesley LJ, Taylor JM, Hesseltine E, Haymart MR, Esfandiari NH, Hanauer DA and Worden FP: Retrospective study of sirolimus and cyclophosphamide in patients with advanced differentiated thyroid cancers. J Thyroid Disord Ther 4: 188, 2015.
33. Hortobagyi GN: Everolimus plus exemestane for the treatment of advanced breast cancer: A review of subanalyses from BOLERO-2. Neoplasia 17: 279-288, 2015.
34. Merli M, Ferrario A, Maffioli M, Arcaini L and Passamonti F: Everolimus in diffuse large B-cell lymphomas. Future Oncol 11: 373-383, 2015.
35. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Figlin RA, Hollaender N, *et al*: Phase 3 trial of Everolimus for metastatic renal cell carcinoma: Final results and analysis of prognostic factors. Cancer 116: 4256-4265, 2010.
36. Kaech SM and Cui W: Transcriptional control of effector and memory $CD8^+$ T cell differentiation. Nat Rev Immunol 12: 749-761, 2012.
37. Nicoli F, Paul S and Appay V: Harnessing the induction of $CD8^+$T-cell responses through metabolic regulation by pathogen-recognition-receptor triggering in antigen presenting cells. Front Immunol 9: 2372, 2018.
38. Amiel E, Everts B, Freitas TC, King IL, Curtis JD, Pearce EL and Pearce EJ: Inhibition of mechanistic target of Rapamycin promotes dendritic cell activation and enhances therapeutic autologous vaccination in mice. J Immunol 189: 2151-2158, 2012.
39. Araki K, Youngblood B and Ahmed R: The role of mTOR in memory CD8 T-cell differentiation. Immunol Rev 235: 234-243, 2010.
40. Nicoli F, Papagno L, Frere JJ, Cabral-Piccin MP, Clave E, Gostick E, Toubert A, Price DA, Caputo A and Appay V: Naïve $CD8^+$ T-cells engage a versatile metabolic program upon activation in humans and differ energetically from memory $CD8^+$ T-Cells. Front Immunol 9: 2736, 2018.
41. Zhang S, Pruitt M, Tran D, Du Bois W, Zhang K, Patel R, Hoover S, Simpson RM, Simmons J, Gary J, *et al*: B cell-specific deficiencies in mTOR limit humoral immune responses. J Immunol 191: 1692-1703, 2013.
42. Nicoli F: Angry, Hungry T-Cells: How Are T-Cell responses induced in low nutrient conditions? Immunometabolism 2: e200004, 2020.
43. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, Rawlings SA, Sutherland A, Premkumar L, Jadi RS, *et al*: Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 181: 1489-1501. e15, 2020.
44. Schulien I, Kemming J, Oberhardt V, Wild K, Seidel LM, Killmer S, Sagar, Daul F, Salvat Lago M, Decker A, *et al*: Characterization of pre-existing and induced SARS-CoV-2-specific $CD8^+$ T cells. Nat Med 27: 78-85, 2021.
45. Gallerani E, Proietto D, Dallan B, Campagnaro M, Pacifico S, Albanese V, Marzola E, Marconi P, Caputo A, Appay V, *et al*: Impaired Priming of SARS-CoV-2-specific naive $CD8^+$ T cells in older subjects. Front Immunol 12: 693054, 2021.
46. Frasca D and Blomberg BB: Inflammaging decreases adaptive and innate immune responses in mice and humans. Biogerontology 17: 7-19, 2016.
47. Keating R, Hertz T, Wehenkel M, Harris TL, Edwards BA, McClaren JL, Brown SA, Surman S, Wilson ZS, Bradley P, *et al*: The kinase mTOR modulates the antibody response to provide cross-protective immunity to lethal infection with influenza virus. Nat Immunol 14: 1266-1276, 2013.
48. Cohen J: Infectious disease. Immune suppressant unexpectedly boosts flu vaccine. Science 342: 413, 2013.
49. Blasi F, Gramegna A, Sotgiu G, Saderi L, Voza A, Aliberti S and Amati F: SARS-CoV-2 vaccines: A critical perspective through efficacy data and barriers to herd immunity. Respir Med 180: 106355, 2021.
50. Cunningham AL, McIntyre P, Subbarao K, Booy R and Levin MJ: Vaccines for older adults. BMJ 372: n188, 2021.
51. Nitulescu GM, Paunescu H, Moschos SA, Petrakis D, Nitulescu G, Ion GND, Spandidos DA, Nikolouzakis TK, Drakoulis N and Tsatsakis A: Comprehensive analysis of drugs to treat SARS-CoV-2 infection: Mechanistic insights into current COVID-19 therapies (Review). Int J Mol Med 46: 467-488, 2020.
52. Gassen NC, Papies J, Bajaj T, Emanuel J, Dethloff F, Chua RL, Trimpert J, Heinemann N, Niemeyer C, Weege F, *et al*: SARS-CoV-2-mediated dysregulation of metabolism and autophagy uncovers host-targeting antivirals. Nat Commun 12: 3818, 2021.
53. Patocka J, Kuca K, Oleksak P, Nepovimova E, Valis M, Novotny M and Klimova B: Rapamycin: Drug repurposing in SARS-CoV-2 infection. Pharmaceuticals (Basel) 14: 217, 2021.