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Research Article

Unlocking Rotavirus Defense: Predicting B Cell Epitopes Targets for Human Rotavirus Type A Vaccines

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Abstract:

Rotavirus, an exceptionally contagious viral agent, holds the distinction of being the primary causal factor behind acute infectious diarrhea in the pediatric demographic under the age of five, thus constituting a profound menace that transcends geographical boundaries. Despite commendable strides in the realm of vaccine development, the terrain of vaccine design and efficacy continues to be rife with challenges. Within the ambit of this investigation, we undertake an exploration of an in-silico avenue for the formulation of rotavirus vaccines, thereby embarking upon a probing analysis of prospective vaccine candidates. The protein sequence of Human Rotavirus Type A was obtained from the UniProt database, and BepiPred 3.0 and ABCpred tools were employed for epitope predictions. Additionally, the secondary structure of RNA molecules for the Human Rotavirus Type A vaccine design was analyzed by utilizing the predictive capabilities of the RNAfold Server. The Class I MHC Immunogenicity and SVMTriP programs were used to predict immunogenicity, while Vaxijen 2.0 was employed to forecast immune properties. According to the results obtained from our study, the CGATGTTGTTGATGGT sequence exhibits epitope potential. This study has provided insights into the analysis of the secondary structure of RNA molecules for the design of Human Rotavirus Type A vaccines, enabling the identification of potential immunogenic regions that could serve as targets for vaccine candidates.

1. Introduction

Rotavirus, a highly contagious virus, is the primary cause of acute infectious diarrhea in children under the age of five across the globe [1-5]. This viral infection poses a significant concern for both developed and developing nations. While it contributes to disease outbreaks in developed countries, it poses a more severe threat in developing nations where it leads to not only illness but also fatalities. The global impact of rotavirus is staggering, causing an estimated 500,000 deaths annually, translating to around 1,600 child deaths every day [6]. To put it another way, a child loses their life due to rotavirus-induced diarrhea approximately every minute worldwide. In developed countries, rotavirus infections result in a cascade of issues, including widespread disease,

hospitalizations, and economic losses. For instance, in the United States, rotavirus-induced diarrhea is responsible for a substantial portion, around 10-12%, of all hospitalizations among children under the age of five [3-6]. The economic ramifications of rotavirus disease are substantial, with estimates suggesting that the financial burden in the United States alone exceeds one billion dollars on an annual basis [7].

Rotavirus infections encompass a wide clinical spectrum, ranging from asymptomatic infection to severe dehydration-inducing fatal gastroenteritis. The initial natural rotavirus infection typically occurs in infants aged 4 to 36 months, manifesting with diarrhea, vomiting, and intense dehydration. Infants usually become immune after 1 to 3 rotavirus infections. Having experienced two prior rotavirus infections provides nearly 100% protection against moderate to severe diarrhea. Hence, the first natural rotavirus infection significantly diminishes the frequency and severity of subsequent infections. The anticipated benefit of rotavirus vaccination is to create immunity similar to natural infection, offering protection against moderate/severe infection, preventing hospitalizations and deaths, reducing illness rates, and minimizing economic losses [8]. Rotavirus vaccines are not expected to provide protection against mild rotaviral diarrhea or recurrent infections [9]. The World Health Organization (WHO) recommended in 2009 that the rotavirus vaccine be included in national vaccination programs globally [10].

Producing human rotaviruses in cell cultures is quite challenging. Despite many unsuccessful attempts, two distinct rotavirus vaccines have been developed and are currently in use.

The in-silico approach for rotavirus vaccines can be employed in areas such as identifying potential vaccine candidates, analyzing antigenic properties, and predicting efficacy. This approach could be significant in cases of emerging new rotavirus strains or for enhancing the broader effectiveness of existing vaccines. The aim of the study is to investigate *in silico* vaccine design against rotavirus and explore the various attributes of the designed vaccine.

2. Material and Methods

2.1 Retrieval of Protein Sequences

Epitope prediction was performed on Rotavirus A variant labeled as Q5IEZ3, sourced from the UniProt protein repository (https://www.uniprot.org/), and subsequently stored in FASTA format to facilitate further examinations.

2.2 Prediction B-Cell Epitopes

BepiPred Version 3.0 was employed to predict potential B cell epitopes within the selected Rota virus Type A protein sequences (https://services.healthtech.dtu.dk/services/BepiPred-3.0/). The BepiPred algorithm utilizes a hidden Markov model to identify linear epitopes based on amino acid propensity scores derived from experimentally validated epitope data. The analysis was performed using default parameters, and predicted epitopes were extracted based on amino acid scores exceeding a predefined threshold [11].

The ABCpred tool was utilized to predict B cell epitopes within the Rotavirus Type A protein sequences (https://webs.iiitd.edu.in/raghava/abcpred/index.html). ABCpred employs an artificial neural network to predict epitope regions by considering the physicochemical properties of amino acids, including antigenicity, hydrophilicity, and surface accessibility. Default parameters were used for the analysis, and predicted epitopes were identified based on the output scores provided by the algorithm [12].

2.3 Prediction The Secondary Structure of RNA Molecule

The RNAfold Server was chosen to be utilized for the vaccine design of Human Rotavirus Type A due to its capability to predict the secondary structure of RNA molecules (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). Since RNA molecules play a crucial role in various biological processes, understanding their secondary structure was considered essential for the design of effective vaccines. The most stable folding pattern of RNA sequences was predicted by the RNAfold Server through the employment of computational algorithms based on thermodynamics and base pairing principles [13]. By the analysis of the secondary structure, insights into potential functional regions, binding sites, and regulatory elements within the RNA molecule could be gained, which could aid in the rational design of vaccines. This approach was found to be particularly valuable for the design of vaccines against Human Rotavirus Type A, where the understanding of the RNA structure could contribute to the development of targeted and efficient vaccine candidates.

2.4 The Immunogenicity Model

The immunogenicity model was predicted through the utilization of the Class I MHC Immunogenicity

(http://tools.iedb.org/immunogenicity) [14] and SVMTriP (A tool to predict Linear Antigenic Epitopes) programs (http://sysbio.unl.edu/SVMTriP) [15]. These tools employed computational algorithms and machine learning techniques to forecast the immunogenicity of peptides within the context of MHC Class I molecules. In this study, the probability of an immune response was assessed by the Class I Immunogenicity program, in particular, through the evaluation of amino acid characteristics and positions within the peptide sequence. VaxiJen is a database designed to assess the likelihood of a specific protein sequence eliciting an immune response and being recognized as an antigen by the immune system. It employs a machine learning approach that takes into account various physicochemical properties of the protein, allowing it to make predictions about its immunogenic potential. In our study, the VaxiJen 2.0 database was utilized for the prediction of Protective Antigens and Subunit Vaccines (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html).

3. Results and Discussions

3.1 Prediction B-Cell Epitopes

The predicted B cell epitopes are ordered based on the scores were generated by a trained recurrent neural network. A greater score for the peptide indicated an increased likelihood of being an epitope. All the peptides presented here surpass the chosen threshold value.
Several epitopes with higher threshold values other than the results provided below have been found. Validation has been conducted. After comparative validation, the best results have been ranked in Table 1 below.

Table 1. *Predicted B-cell Epitopes*

Sequence	Start Position	Score
ACTCAAATTGGAGATA	184	0.94
GACAGTCTTGTACCAT	476	0.93
ACTTTAGGAATAGGTT	517	0.91
TTCAACGTTAGAGCTA	348	0.91
TACGGAATGGAAGGAT	198	0.91
AGATGTGTTAGATATT	711	0.90
CTTATTGTTATTGCAT	31	0.90
CGATGTTGTTGATGGT	594	0.89

Table 2. *Predicted B-cell epitopes are shown using BcePred Prediction.*

Hydrophilicity	TTTCTTTT, TTATTGTTATTG, CTTTTGTTA, ATTTTTGA, ATTGGAGAT, GGAATGGAAGG, AAGGGTGGC, TTTATTGTGATT, ATGTTGTA, GTTAGAG, TGAATGGTTATGT, GATGGGA, GTCTTGT, TAGGTTGTATT, AGAGGTGGCT, GATGTTGTTGATGGTGTGAA, TTGATGTGA, GGAATTG, AGTTAGGA, GTCGGTGGCT, AGATGTGTTAG
Flexibility	AAAGGGT, ATAGGTT, TGATGGTG
Antigenic Propensity	CTGGCTCC, CTCTGTCCC, TCGCTTC

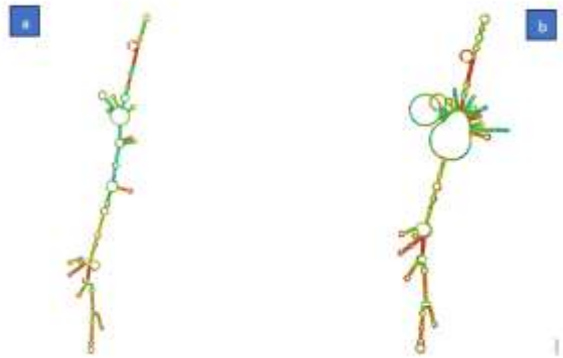

Figure 1. *(A) Minimum Free Energy (MFE) secondary structure colored by base-pairing probability. (B) Centroid secondary structure showing the most probable folding. Unpaired regions in red indicate high likelihood of being unpaired; paired regions in green indicate stable base-pairing.*

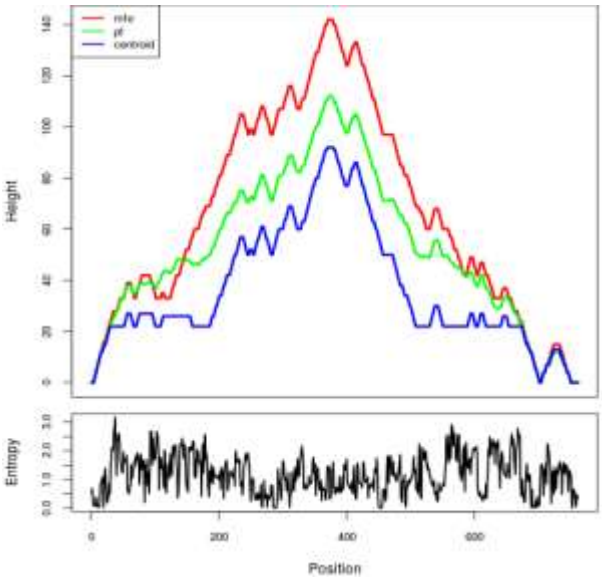

Figure 2. *Mountain plot representation of the predicted RNA structure: showing the MFE structure (blue), thermodynamic ensemble (green), and centroid structure (red). The entropy values reflect structural variability at each nucleotide position.*

Table 2 demonstrates that the provided information presents the predicted B-cell epitopes as determined by the BcePred Prediction tool. The data presented encompasses various sequences that display potential antigenic characteristics, identified through distinct criteria such as hydrophilicity, flexibility, and antigenic propensity.

The results of the thermodynamic ensemble prediction are intriguing. The calculated free energy of the thermodynamic ensemble at -176.79 kcal/mol suggests a potentially stable RNA structure. However, it's notable that the frequency of the Minimum Free Energy (MFE) structure within the ensemble is reported as 0.00%, which raises questions about the prevalence of this specific structure. The ensemble diversity value of 203.53 further indicates the variability and complexity of the RNA conformations present. These results prompt further investigation into the underlying factors contributing to the ensemble composition and the implications for the RNA's biological function. The mountain plot of the MFE structure, the thermodynamic ensemble of RNA structure was shown in Figure 2.

Table 3. *MHC Class I Immunogenicity Peptide Length Score*

Peptide	**Length**	**Score**
AGATGTGTTAGATATT	16	0.4326
ACTTTAGGAATAGGTT	16	0.42884
CGATGTTGTTGATGGT	16	0.425
TACGGAATGGAAGGAT	16	0.38916
CTTATTGTTATTGCAT	16	0.35913
ACTCAAATTGGAGATA	16	0.33995
TTCAACGTTAGAGCTA	16	0.23435
GACAGTCTTGTACCAT	16	0.15128

Table 3 provides a detailed presentation of the MHC Class I Immunogenicity results. Each peptide sequence is listed with its length and score specified. These scores reflect the potential interaction of peptides with MHC Class I molecules. The scores are based on calculations to assess the likelihood of triggering a possible immune response. The table has presented the immunogenicity values predicted by the MHC Class I Immunogenicity database for these peptide sequences.

An overall prediction value of 0.5674 for the Protective Antigen is indicated by the provided VaxiJen 2.0 results, suggesting a likelihood of the antigen being probable. This prediction is in alignment with the potential immunogenicity of the sequence, thereby highlighting its potential as a candidate for further investigation in the realms of vaccine development or immunological research.

In the realm of human rotavirus research, cultivating these viruses in cell cultures has posed significant challenges [16]. Despite these hurdles, the development of two distinct rotavirus vaccines that are currently in use stands as a remarkable achievement [17]. However, an alternative avenue for advancing rotavirus vaccine development has emerged through the utilization of in-silico methods [18]. These computational tools hold promise in identifying potential vaccine candidates, analyzing antigenic properties, and predicting the efficacy of vaccines. This approach presents a particularly valuable opportunity to address emerging rotavirus strains and enhance the overall effectiveness of existing vaccines [19]. With these considerations in mind, the present study aims to explore the potential of in-silico vaccine design against rotavirus, delving into the multifaceted attributes of the designed vaccine candidates [20].

A comparison with current rotavirus vaccine formulations, such as *Rotarix™* and *RotaTeq™*, reveals that these vaccines primarily utilize attenuated virus strains targeting the outer capsid proteins VP4 and VP7. In contrast, the present study identifies novel linear B-cell epitope candidates—such as *CGATGTTGTTGATGGT*—using *in silico* tools, independent of whole-virus constructs. These epitopes may offer enhanced specificity and adaptability, especially in peptide- or subunit-based vaccine strategies [21-24]. This approach is particularly valuable given the antigenic variation observed in circulating rotavirus strains, which can challenge the cross-protection breadth of existing vaccines. At the core of this investigation is the prediction of B cell epitopes – specific segments within proteins or genetic sequences that can interact with antibodies produced by B cells. This interaction plays a pivotal role in the immune response. In the context of this study, the functionality of the CGATGTTGTTGATGGT sequence as an epitope has been thoroughly analyzed. This analysis sheds light on the potential antigenic characteristics of this sequence, offering insights into its viability as a component in vaccine development or as a subject for immunological research. However, it's worth

noting that, like other computational tools, while VaxiGen 2.0 and epitope prediction provide valuable initial screening, experimental validation through wet laboratory experiments remains essential to ascertain the practical immunogenicity of the identified sequence.

The immune system's orchestration heavily relies on MHC (Major Histocompatibility Complex) Class I molecules, which play a pivotal role in presenting peptides from intracellular proteins to cytotoxic T-cells. This presentation influences immune responses by categorizing peptides as "self" or "non-self," impacting cytotoxic T-cell reactions against infected or cancerous cells [25].

In this context, the MHC Class I Immunogenicity prediction program becomes a valuable tool for researchers to predict the probability of a given peptide sequence being recognized by the immune system and triggering an immune response. Our attempt to validate our analyses' outcomes from tools like ABCpred and BepiPred was executed through the utilization of the MHC I Immunogenicity program. This validation process revealed that the obtained results are consistent with the existing data. Such outcomes hold applications across diverse domains including vaccine design, drug development, and personalized medicine. The insights derived from the MHC I Immunogenicity program can pave the way for innovative approaches and more effective solutions in these fields, playing a pivotal role in comprehending immune responses to diseases and shaping potential treatment strategies. The Class I Immunogenicity program scrutinizes peptide sequences, assessing their interactions with MHC Class I molecules. This analysis takes into account factors such as peptide binding affinity, stability, and antigen presentation efficiency. By considering these facets, the program predicts whether a given peptide sequence is likely to initiate an immune response by engaging with cytotoxic T-cells. On a parallel note, the SVMTrip program employs machine learning to differentiate between immunogenic and non-immunogenic peptides. It factors in properties like amino acid physicochemical attributes, sequence motifs, and binding affinity to MHC molecules. The program's training data is derived from labeled peptide sequences with known immunogenicity status, enabling the SVM algorithm to learn the distinctive patterns associated with immunogenic peptides.

4. Conclusions

This study utilized in-silico methods to identify potential B-cell epitopes and evaluate RNA secondary structures for the development of Human Rotavirus Type A vaccines. The key findings include the identification of the CGATGTTGTTGATGGT sequence as a promising epitope, alongside predictions of potential immunogenicity and stability of RNA structures. These findings are significant as they offer new insights into the design of targeted vaccines and enhance our understanding of rotavirus immunogenicity. However, the study's limitations include reliance on computational predictions without experimental validation and the need for further investigation into the practical effectiveness of identified epitopes. Future research should focus on experimental validation of these findings, exploration of additional epitope candidates, and optimization of RNA-based vaccine designs to improve vaccine efficacy and protection against emerging rotavirus strains.

While the study offers novel insights through robust *in silico* methodologies, its scientific contribution remains preliminary in the absence of experimental validation. Future work is essential to confirm the immunogenic potential of identified epitopes through *in vitro* and *in vivo* experiments

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- **Ethical approval:** The conducted research is not related to either human or animal use.
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References

[1] Parashar, U. D., Hummelman, E. G., Bresee, J. S., Miller, M. A., & Glass, R. I. (2003). Global illness and deaths caused by rotavirus disease in children. *Emerging infectious diseases*, 9(5), 565–572. https://doi.org/10.3201/eid0905.020562

[2] Girish Kumar, C. P., Giri, S., Chawla-Sarkar, M., Gopalkrishna, V., Chitambar, S. D., Ray, P., et al. (2020). Epidemiology of rotavirus diarrhea among children less than 5 years hospitalized with acute gastroenteritis prior to rotavirus vaccine introduction in India. *Vaccine*, 38(51), 8154–8160. https://doi.org/10.1016/j.vaccine.2020.10.084

[3] Chissaque, A., Cassocera, M., Gasparinho, C., Langa, J. S., Bauhofer, A. F. L., Chilaúle, J. J et al. (2021). Rotavirus A infection in children under five years old with a double health problem: undernutrition and diarrhoea - a cross-sectional study in four provinces of Mozambique. *BMC infectious diseases*, 21(1), 18. https://doi.org/10.1186/s12879-020-05718-9

[4] Gbebangi-Manzemu, D., Kampunzu, V. M., Vanzwa, H. M., Mumbere, M., Bukaka, G. M., Likele, B. B. et al. (2023). Clinical profile of children under 5 years of age with rotavirus diarrhoea in a hospital setting in Kisangani, DRC, after the introduction of the rotavirus vaccine, a cross-sectional study. *BMC pediatrics*, 23(1), 193. https://doi.org/10.1186/s12887-023-04022-0

[5] Murphy, A., Kirby, A., & De Blasio, F. (2023). The economic impact of the introduction of universal rotavirus vaccination on rotavirus gastroenteritis related hospitalisations in children in Ireland. *Vaccine*, 41(16), 2656–2663. https://doi.org/10.1016/j.vaccine.2023.03.010

[6] Bresee, J. S., Parashar, U. D., Widdowson, M. A., Gentsch, J. R., Steele, A. D., & Glass, R. I. (2005). Update on rotavirus vaccines. *The Pediatric infectious disease journal*, 24(11), 947–952. https://doi.org/10.1097/01.inf.0000186295.18969.e6

[7] Glass, R. I., Kilgore, P. E., Holman, R. C., Jin, S., Smith, J. C., Woods, P. A. et al.(1996). The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. *The Journal of infectious diseases*, 174 Suppl 1, S5–S11. https://doi.org/10.1093/infdis/174.supplement_1.s5

[8] Velázquez, F. R., Matson, D. O., Calva, J. J., Guerrero, L., Morrow, A. L., Carter-Campbell, S., et al. (1996). Rotavirus infection in infants as protection against subsequent infections. *The New England journal of medicine*, 335(14), 1022–1028. https://doi.org/10.1056/NEJM199610033351404

[9] Rappuoli, R., Pizza, M., Covacci, A., Bartoloni, A., Nencioni, L., Podda, A., & De Magistris, M. T. (1992). Recombinant acellular pertussis vaccine--from the laboratory to the clinic: improving the quality of the immune response. *FEMS microbiology immunology*, 5(4), 161–170. https://doi.org/10.1111/j.1574-6968.1992.tb05898.x

[10] Rotavirus vaccines WHO position paper: January 2013 - Recommendations. (2013). *Vaccine*, 31(52), 6170–6171. https://doi.org/10.1016/j.vaccine.2013.05.037

[11] Jespersen, M. C., Peters, B., Nielsen, M., & Marcatili, P. (2017). BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic acids research*, 45(W1), W24–W29. https://doi.org/10.1093/nar/gkx346

[12] Malik, A. A., Ojha, S. C., Schaduangrat, N., & Nantasenamat, C. (2022). ABCpred: a webserver for the discovery of acetyl- and butyryl-cholinesterase inhibitors. *Molecular diversity*, 26(1), 467–487. https://doi.org/10.1007/s11030-021-10292-6

[13] Gruber, A. R., Lorenz, R., Bernhart, S. H., Neuböck, R., & Hofacker, I. L. (2008). The Vienna RNA websuite. *Nucleic acids research*, *36(Web Server issue)*, W70–W74. https://doi.org/10.1093/nar/gkn188

[14] Tung, C. W., & Ho, S. Y. (2007). POPI: predicting immunogenicity of MHC class I binding peptides by mining informative physicochemical properties. *Bioinformatics (Oxford, England)*, 23(8), 942–949. https://doi.org/10.1093/bioinformatics/btm061

[15] Yao, B., Zhang, L., Liang, S., & Zhang, C. (2012). SVMTriP: a method to predict antigenic epitopes using support vector machine to integrate tri-peptide similarity and propensity. *PloS one*, 7(9), e45152. https://doi.org/10.1371/journal.pone.0045152

[16] Albert, M. J., & Bishop, R. F. (1984). Cultivation of human rotaviruses in cell culture. *Journal of medical virology*, 13(4), 377–383. https://doi.org/10.1002/jmv.1890130409

[17] Burke, R. M., Tate, J. E., Kirkwood, C. D., Steele, A. D., & Parashar, U. D. (2019). Current and new rotavirus vaccines. *Current opinion in infectious diseases*, 32(5), 435–444. https://doi.org/10.1097/QCO.0000000000000572

[18] Ndwandwe, D., Runeyi, S., Mathebula, L., & Wiysonge, C. (2022). Rotavirus vaccine clinical trials: a cross-sectional analysis of clinical trials registries. *Trials*, 23(1), 945. https://doi.org/10.1186/s13063-022-06878-6

[19] Saba, A. A., Adiba, M., Saha, P., Hosen, M. I., Chakraborty, S., & Nabi, A. H. M. N. (2021). An in-depth in silico and immunoinformatics approach for designing a potential multi-epitope construct for the effective development of vaccine to combat against SARS-CoV-2 encompassing variants of concern and interest. *Computers in biology and medicine*, 136, 104703. https://doi.org/10.1016/j.compbiomed.2021.104703

[20] Usman, M., Ayub, A., Habib, S., Rana, M. S., Rehman, Z., Zohaib, A., Jamal, S. B., Jaiswal, A. K., Andrade, B. S., de Carvalho Azevedo, V., Faheem, M., & Javed, A. (2023). Vaccinomics Approach for Multi-Epitope Vaccine Design against Group A Rotavirus Using VP4 and VP7

Proteins. *Vaccines*, 11(4), 726. https://doi.org/10.3390/vaccines11040726

[21] Dennehy P. H. (2008). Rotavirus vaccines: an overview. *Clinical microbiology reviews*, *21*(1), 198–208. https://doi.org/10.1128/CMR.00029-07

[22] Burnett, E., Jonesteller, C. L., Tate, J. E., Yen, C., & Parashar, U. D. (2017). Global Impact of Rotavirus Vaccination on Childhood Hospitalizations and Mortality From Diarrhea. *The Journal of infectious diseases*, *215*(11), 1666–1672. https://doi.org/10.1093/infdis/jix186

[23] Keating G. M. (2006). Rotavirus vaccine (RotaTeq). *Paediatric drugs*, *8*(3), 197–204. https://doi.org/10.2165/00148581-200608030-00008

[24] Buyse, H., Vinals, C., Karkada, N., & Han, H. H. (2014). The human rotavirus vaccine Rotarix™ in infants: an integrated analysis of safety and reactogenicity. *Human vaccines & immunotherapeutics*, *10*(1), 19–24. https://doi.org/10.4161/hv.26476

[25] Pishesha, N., Harmand, T. J., & Ploegh, H. L. (2022). A guide to antigen processing and presentation. *Nature reviews. Immunology*, 22(12), 751–764. https://doi.org/10.1038/s41577-022-00707-2