

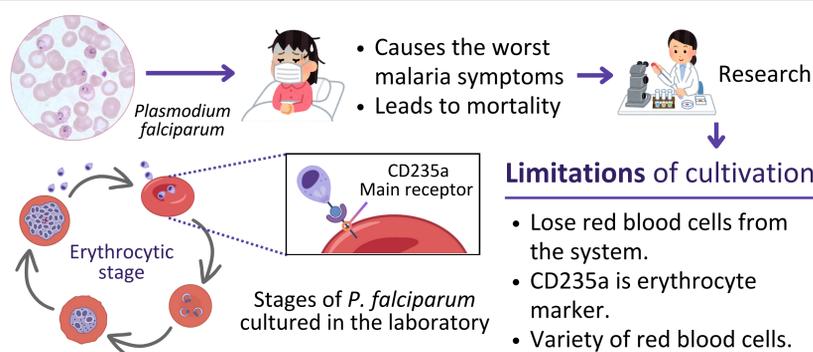
## Abstract

*Plasmodium falciparum* is one of the *Plasmodium* species that causes malaria symptoms in humans and is one of the most extensively studied to date. *Plasmodium falciparum* parasites have a specific host: human red blood cells. This specificity limits the ability to culture the parasite for laboratory studies. The objective of this study is to develop cells that can replace the use of red blood cells in the cultivation of *Plasmodium falciparum* by increasing the expression of the CD235a protein (the main receptor for the parasite's invasion) and stimulating hemoglobin production in the K562 human erythroleukemia cell line (K562 cell line). Following co-cultivation with *Plasmodium falciparum*, the percentage of parasitemia in the cells will be evaluated at three different time points: 24, 45, and 96 hours. The studies showed that the K562 cell line, when enhanced with the expression of the CD235a protein, could produce hemoglobin at a rate of  $7.02 \pm 4.14$  %. In the results of the percentage of parasitemia, it was found that K562 cells in the hemoglobin-stimulated group showed a trend of increased invasion rates at all time points during the experiment. Particularly at the 96-hour time point, K562 cells overexpressing CD235a in the hemoglobin-stimulated group had a higher percentage of parasitemia than those without CD235a overexpression in the same group. Although the difference was not statistically significant, the higher invasion rate in K562 cells with CD235a overexpression compared to those without overexpression still indicates a positive sign of *Plasmodium falciparum* invasion in K562 cells. The results of this study provide data and insights for developing more suitable cells to replace red blood cells in future studies.

## Objective

To develop a host to replace human erythrocytes for *Plasmodium falciparum* propagation via studying the CD235a overexpressed K562 cell line and stimulating the production of hemoglobin using hemin.

## Introduction

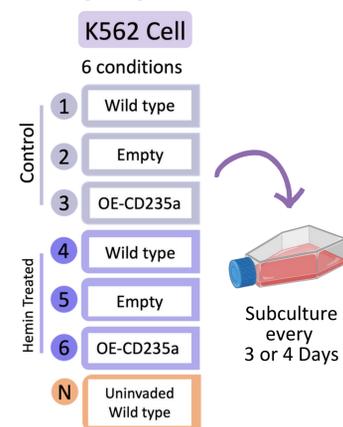


## K562 cell line

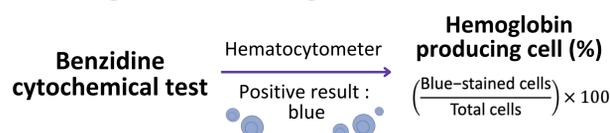
- A cell line that can be developed into red blood cells
- CD235a can be found on the cell surface.
- It stimulates the formation of hemoglobin by using Hemin.

## Methodology

### Cell preparation



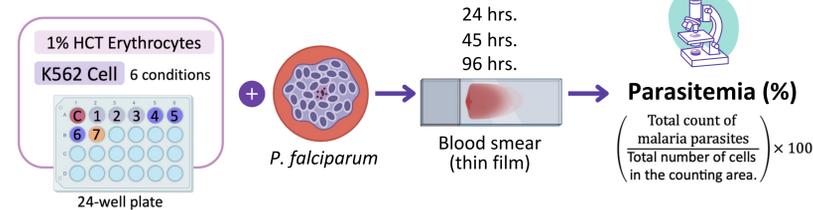
### Hemoglobin Testing



### Statistical Test

jamovi, R Studio

### *P. falciparum* Invasion Test



## Results and discussion

### Preliminary studies

In 2021, Jaitan studied the CD235a overexpression in K562 cell line by using the following molecular techniques: Immunofluorescence, Flow cytometry, and Immunoblot analysis; and confirmed that there is the expression in the cells.

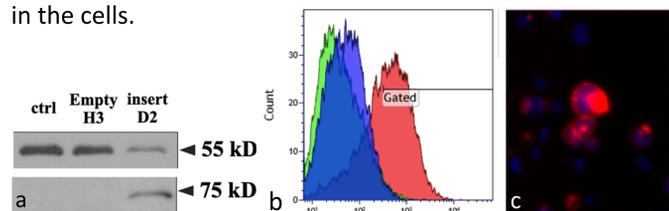


Figure 1: Illustrations of a) immunoblot analysis b) flow cytometry and c) immunofluorescence

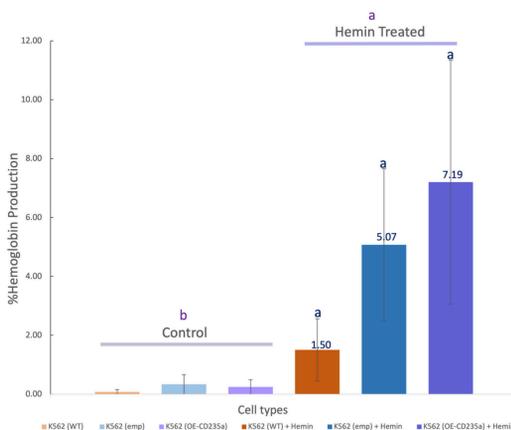


Figure 2: The graph shows the percentage of cells producing hemoglobin.

### Hemoglobin Testing

The graph indicates that the cells in the hemin-treated group produce significantly higher levels of hemoglobin compared to the cells in the control group. Additionally, the color of the cell pellet in the Hemin-treated group began to change, becoming more red, whereas in the control group, the cell pellet remained white. The results obtained are consistent with the research of Pomlok (2019) which can stimulate K562 cells to produce hemoglobin using hemin.

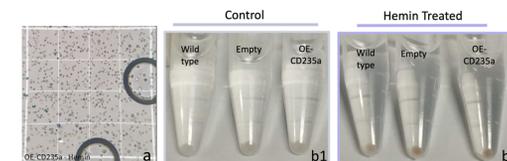


Figure 3: Illustrations of a) The blue staining of hemoglobin-producing cells from the Benzidine cytochemical test. b) Cell pellets: b1: control group and b2: Hemin-treated group

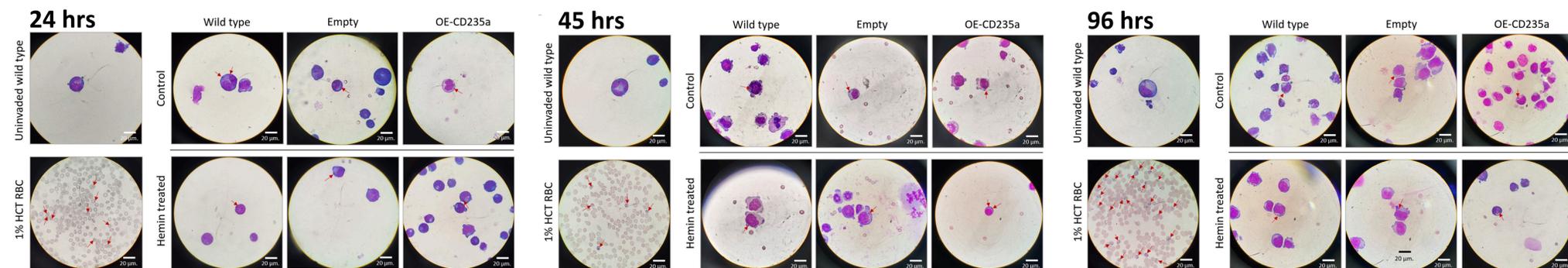


Figure 4: Illustrations of the invasion of *P. falciparum* in red blood cells and all cells under six conditions at three time points: 24, 45, and 96 hours.

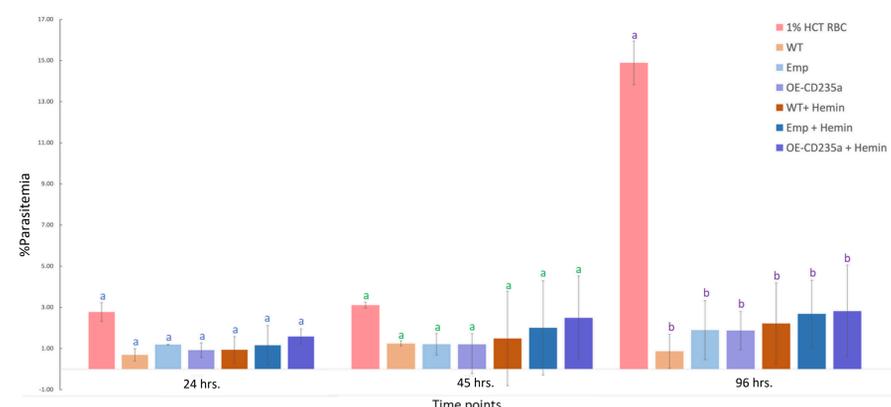


Figure 5: The graph shows the percentage of invasion by *P. falciparum* at three time points: 24, 45, and 96 hours.

### *P. falciparum* Invasion Test

- The graph indicates that at 24 hours, the parasite invasion percentage does not differ significantly. However, at 45 and 96 hours, the hemin-treated cell group shows an increased invasion percentage.
- At 96 hours, parasite invasion in red blood cells was highest and significantly different across all six conditions.

The observed results are likely due to stress from subculture and co-culture with hemin, which is toxic to K562 cells at high concentrations, causing ROH accumulation.

## conclusion

In the research that K562 cells can be induced to synthesize hemoglobin by using hemin. Moreover, in assessing *Plasmodium falciparum* invasion, it was observed that the parasite was able to invade the cells. In addition, the results of the percentage of parasitemia, K562 cells with CD235a overexpression showed a higher invasion rate compared to those without overexpression, indicating a positive sign of *Plasmodium falciparum* invasion in K562 cells.

