**Proteasome inhibition to treat triple negative breast cancer:**

**A Novel Molecular Mechanism for the Inhibition of the 26S Proteasome by disassembly of the 19S particle**

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| **Project Number:** | 1619 |
| **Principal Investigator:** | Prof. Yosef Shaul |
| **Patent Status:** | Pending |

**Overview**

Triple Negative Breast Cancer (TNBC) is an aggressive and lethal type of breast cancer, representing 10%-20% of all cases. There is no clinically approved therapy that directly targets and effectively treats TNBC. The standard action is to use chemotherapy for the entire body in the hope that the generalized treatment will be effective. However, TNBC is known for the high chance of relapse and being terminal in its metastasized form. **Subsequently there is a strong need for a specific and effective therapy targeting TNBC**.

The present innovation is a unique target and mechanism in treating triple negative breast cancer cells. The technology functions by inhibiting the 26S proteasome, a crucial component in maintaining a cell. However, rather than targeting the 26S proteasome’s 20S particle, which is the common approach, the technology targets the regulatory 19S particle. This leads to an innovative target for a difficult to treat form of breast cancer.

**The Unmet Need**

Triple negative breast cancer is known for a low survival rate, high probability of recurrence, and metastasis is nearly assured to lead to death within a short timeframe. Consequently this makes TNBC a particularly fatal form of cancer. What distinguishes TNBC is that it lacks the estrogen, progesterone, and HER2 receptors, commonly found in other forms of breast cancer. Subsequently traditional hormone and HER2 directed therapies are ineffective against TNBC. Therefore the standard treatment for TNBC is the use of systemic (body-wide) chemotherapy; however this action has shown limited success.

**Accordingly there is a strong need to discover potential targets and generate therapeutics that is specifically directed against triple negative breast cancer cells.**

**The Solution**

The research team of Prof. Yosef Shaul has discovered a unique target in terms of treating triple negative breast cancer. The innovation is the inhibition of the critical cell maintaining element, the 26S proteasome, except that it uses the distinct mechanism of inducing disassembly of the 19S regulatory particle by knocking down its PSMD1 subunit, rather than the more common 20S particle targeting. This has the effect of selectively inhibiting 26S activity, but leaving proteasomal pathways dependent solely on the 20S particle active.

**Technology Essence:**

The foundation of the technology is that 26S proteasome inhibition is known to be an effective treatment for numerous types of cancers. The 26S proteasome is composed of the 20S particle, a proteolytic catalytic unit, and one or two 19S particles that act as regulatory units to process and translocate ubiquitinated proteins to the 20S catalytic unit. Normally inhibition of the 26S proteasome targets the 20S particle to directly inhibit the catalytic activity. However, the technology of the Shaul group targets the 19S particle(s), while leaving the 20S particle intact.

The technology functions by the use of shRNA to knockdown a subunit of the 19S particle, PSMD1 (Rpn2), which causes the disassembly of the 19S particle and loss of the 26S activity. This was demonstrated by transducing via lentivirus the non-tumorigenic MCF10A cell line with the shRNA to knockdown the Psmd1 subunit. Following the cells were either left untransformed (naïve) or transformed with the Ras oncogene. The shRNA was then induced by doxycycline in both the untransformed and transformed cells. In Figure 1A, a western blot shows that the Ras oncogene causes a significant expression of Psmd1, signifying an increased expression of the 26S proteasome. However, induction of the shRNA leads to a reduced expression of Psmd1 (Fig. 1A). This was then further examined by looking at 26S proteasome activity in native PAGE (Fig. 1B). Again the Ras oncogene transformed cells sans shRNA induction show a high proteasomal activity. Though once Psmd1 is knocked down, there is a dramatic reduction in the activity of the 26S proteasome activity.



Figure 1: Knocking down Psmd1 reduced 26S proteasome activity in Ras oncogene transformed cells. Non-tumorigenic MCF10A cell line was transduced with doxycycline inducible Psmd1 shRNA. Cells were left untransformed (naïve) or transformed with the Ras oncogene. A) Western blot displaying protein expression of Psmd1 with actin staining as a loading control. B) 26S proteasome activity in native PAGE, DC is double capped (two 19S units), SC is single capped (one 19S unit), bound to the 20S particle.

The technology was additionally applied to a drug resistant triple negative breast cancer (TNBC) cell line MD-MB-231. The MD-MB-231 cells were transduced by lentivirus to include a doxycycline inducible Psmd1 shRNA. The cells were then tested to determine their proliferation rate, with and without Psmd1 knockdown (Fig. 2). In the case of TNBC cell MD-MB-231, there was only growth when there was no Psmd1 knockdown (Fig. 2A). Compared to the control MCF10A cells, Psmd1 knockdown was tolerated well by the cells (Fig. 2B).



Figure 2: Triple Negative Breast Cancer Cell line is sensitive to Psmd1 knockdown. Cell lines were transduced with doxycycline inducible Psmd1 shRNA and proliferation rates were observed. A) MD-MB-231 is a triple negative breast cancer cell line that is drug resistant. B) MCF10A is a non-tumorigenic cell line.

**Applications:**

* Generating therapeutics towards Triple Negative Breast Cancer.

**Advantages:**

* **Specific** – the technology explicitly targets triple negative breast cancer cells.
* **Unique mechanism** – inducing disassembly of the 19S particle, by explicitly knocking down Psmd1 subunit of the 19S complex leads to 26S proteasome inhibition, but leaves 20S activity unperturbed.
* **Flexible drug delivery** – system has been shown to be effective using Lentiviral delivery and could possibly use standard RNA interference chemistries.

**Development Status**

Researchers from the group of Prof. Shaul at the Weizmann Institute have developed a novel therapeutic target for triple negative breast cancer. The knockdown of the Psmd1 subunit of the 19S particle of the 26S proteasome was shown effective in a cell displaying tumorigenic qualities and a drug resistant triple negative breast cancer cell line.

**Market Opportunity**

Triple negative breast cancer (TNBC) represents approximately 10-20% of breast cancer cases. This form of breast cancer is aggressive, with a poor survival prognosis, high probability of recurrence, and essentially becomes fatal if it metastasizes.

There is an absence of specifically targeted therapeutics for TNBC, leading to the use of a generalized chemotherapy regimen. However, chemotherapy has not shown itself to be the most effective option. Thus there is a market opportunity for treating TNBC.

This technology of inhibiting the 26S proteasome by knocking down the Psmd1 subunit of the 19S particle, and leading to its disassembly is a novel option in treating TNBC. The technology has already been shown deployable in lentiviral vector and could use standard RNA interference chemistries. Finally the technology has been shown to effectively stop proliferation of cell lines induced to be tumorigenic and a drug resistant TNBC cancer cell line, while being tolerated by non-tumorigenic cells.

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