
# Cutting the brake lines: Unlocking cancer immunity?

**Educator guide**

## PAPER DETAILS

**Original title:** Enhancement of Antitumor Immunity by CTLA-4 Blockade

**Reference:** Vol. 271, Issue 5256, pp. 1734-1736

**Authors:** Dana R. Leach, Matthew F. Krummel, James P. Allison

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# DISCUSSION QUESTIONS

| | LEARNING STANDARDS |
|---|---|
| 1. What was the understanding of the role of CTLA-4 on T-cell activation at the time this paper was published? Did the authors' conclusions agree or disagree with this understanding? | SEP4<br>RST.11-12.2<br>RST.11-12.8<br>EK3.D.3<br>NS5 |
| 2. What are other potential reasons for the regression of the tumors in mice? How do the control groups address those possibilities? | SEP3<br>Cause and effect<br>SP3<br>EK2.D.1 |
| 3. Why do you think it is important for an animal's immune system to have checkpoints, such as CTLA-4? | SEP1<br>LS1.A<br>LS3.B<br>Structure and function<br>EK2.D.4 |
| 4. Beyond the data shown in this study, what other lines of evidence would be necessary to collect before using anti–CTLA-4 as an antitumor treatment? | SEP3<br>RST.11-12.6<br>VC6<br>NS2 |
| 5. Read the overview of the drug development process as outlined by the FDA in this resource. https://www.fda.gov/patients/learn-about-drug-and-device-approvals/drug-development-process<br>Which stage of the process was this paper trying to contribute to? | SEP1<br>RST.11-12.8<br>VC6 |

# ACTIVITIES FOR INTERACTIVE ENGAGEMENT

| | LEARNING STANDARDS |
|---|---|
| **Writing an abstract**<br><br>Students write a new abstract for the article at a grade-appropriate reading level. | RST.9-10.2<br>RST.11-12.2<br>NS2 |
| **Locating this study in the larger field**<br><br>Students use the annotated list of references to explain how this research builds on the published work of at least one other independent group of scientists. Students will evaluate whether data from this research supports or contradicts previous conclusions and reflect on the statement that scientific knowledge is a "community effort." | RST.9-10.8<br>RST.11-12.8<br>NS5 |
| **Science in the news**<br><br>Students explore news stories in the Related Resources tab and evaluate the stories for tone, accuracy, missing information, etc. They may then write their own news stories on the article. | RST.9-10.5<br>RST.11-12.5<br>RST.9-10.6<br>RST.11-12.6<br>RST.9-10.8<br>RST.11-12.8 |
| **Data representation**<br><br>Students represent the data provided in the paper's figures in a different format. For example, students could create a data table, with columns such as number of mice in a group, tumor load, type of treatment given, time at which treatment was given, and average tumor growth or tumor regression. Students may then discuss the advantages and disadvantages of different kinds of graphical data representation. | SEP4<br>Systems and system models<br>VC2<br>NS2 |
| **Results and conclusions**<br><br>Students diagram each of the experiments presented in the study (divided up by figure, if appropriate). They then consider the results depicted in each figure and how these results support the conclusions of the study. | SEP4<br>Cause and effect<br>SP1<br>VC2 |
| **The next steps**<br><br>Students design a follow-on experiment to this study that either addresses flaws or unanswered questions in the research at hand or builds on it to explore a new question. | SEP3<br>Systems and system models<br>SP3<br>NS5 |

# ARTICLE OVERVIEW

**Article summary (recommended for educator use only)**

The authors investigate the effect of blocking CTLA-4, a T-cell inhibition signaling receptor, on the ability of the immune system to fight tumors in a mouse study. They find that blocking the CTLA-4 receptor allows the mice to more rapidly shrink the tumor size in a variety of experiments, compared to no treatment or an irrelevant antibody control. This was the case for B7-positive colon carcinomas, B7-negative colon carcinomas, tumors pre-established prior to treatment, and fibrocarcinomas. The treatment additionally allows the mice to develop a memory response against subsequent exposure to the same tumors. This is the seminal paper in the work of 2018 Nobel Prize winner James Allison on immune checkpoint therapies.

**Importance of this research**

The immune system is exquisitely effective at fighting off many infections, but tumors are often able to evade it. Addressing the core mechanisms by which tumors can evade recognition by T cells could allow the development of a catch-all treatment that could work regardless of the specific type of cancer that a patient has. The identification of CTLA-4 as an attractive target for these studies is based on its role as a T-cell inhibitor. The authors here show that removing this inhibition allows T cells to activate and proliferate much more. It remains to be seen the exact mechanisms by which anti-CTLA-4 can elicit antitumor immunity, and the safety and effectiveness of this treatment in humans, as well as the combinations of treatments it can be used with.

**Experimental methods**

- Protein G purification of antibodies: Antibodies were produced by hybridomas and then purified from the growth media by utilizing the specific protein interaction of antibody Fc fragment with protein G.
- Cloning and transfection of DNA: The DNA sequence encoding the gene for murine B7-1 was manipulated using standard cloning methods and transfected into tumor cells.
- Mammalian cell culture: Cultures of tumor cells were maintained, passaged, and counted in vitro.
- Flow cytometry: B7-1 molecules were stained with specific antibody markers and the level of staining analyzed by flow cytometry to probe expression of B7-1 on the surface of cells.
- Subcutaneous and intraperitoneal injection of mice: Tumors were established in mice by subcutaneous (under the skin) injection, and treatments were given by intraperitoneal (within the membrane lining of the abdominal cavity) injection.

**Conclusions**

- Blocking CTLA-4 using an antibody enhances tumor rejection.
    - This treatment is also effective for tumors, which are established a few days before the treatment is given.
    - The immunity elicited by this treatment results in a memory response against secondary exposure to tumor cells.
    - This was true for murine colon carcinomas, as well as more aggressive murine fibrocarcinomas, regardless of whether the tumors were engineered or wild-type.

# LEARNING STANDARDS ALIGNMENT

The following tables provide an overview of the learning standards covered by this article, including A Framework for K-12 Science Education (Framework), Common Core State Standards English Language Arts & Literacy (CCSS ELA), Common Core State Standards Statistics and Probability (CCSS HSS), AP Science Practices, and Vision and Change in Undergraduate Biology Education. Where applicable, activities and information will be marked with specific standards to which they are linked.

| A Framework for K-12 Science Education | | |
|---|---|---|
| **Science and Engineering Practices** | **Disciplinary Core Ideas** | **Crosscutting Concepts** |
| **Asking Questions and Defining Problems (SEP1)**<br>Ask questions that arise from examining models or a theory, to clarify and/or seek additional information and relationships.<br><br>**Planning and Carrying Out Investigations (SEP3)**<br>Consider possible confounding variables or effects and ensure that the investigation's design has controlled for them.<br><br>**Analyzing and Interpreting Data (SEP4)**<br>Consider limitations of data analysis (e.g., measurement error, sample selection) when analyzing and interpreting data. Evaluate the impact of new data on a working explanation and/or model of a proposed process or system. | **LS1.A: Structure and function**<br>Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range.<br><br>**LS3.B: Variation of traits**<br>Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation. | **Cause and effect**<br>Events have causes, sometimes simple, sometimes multifaceted. A major activity of science is investigating and explaining causal relationships and the mechanisms by which they are mediated. Such mechanisms can then be tested across given contexts and used to predict and explain events in new contexts.<br><br>**Systems and system models**<br>Defining the system under study—specifying its boundaries and making explicit a model of that system—provides tools for understanding and testing ideas that are applicable throughout science and engineering.<br><br>**Structure and function**<br>The way in which an object or living thing is shaped and its substructure determine many of its properties and functions. |

| Common Core State Standards English Language Arts & Literacy | | |
|---|---|---|
| Key Ideas and Details | Craft and Structure | Integration of Knowledge and Ideas |
| **RST.9-10.1**<br>Cite specific textual evidence to support analysis of science and technical texts, | **RST.9-10.4**<br>Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or | **RST.9-10.8**<br>Assess the extent to which the reasoning and evidence in a text support the author's claim |

attending to the precise details of explanations or descriptions.

**RST.9-10.2**
Determine the central ideas or conclusions of a text; trace the text's explanation or depiction of a complex process, phenomenon, or concept; provide an accurate summary of the text.

**RST.11-12.1**
Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.

**RST.11-12.2**
Determine the central ideas or conclusions of a text; summarize complex concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.

technical context relevant to grades 9-10 texts and topics.

**RST.9-10.5**
Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).

**RST.9-10.6**
Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, defining the question the author seeks to address.

**RST.11-12.4**
Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11-12 texts and topics.

**RST.11-12.5**
Analyze how the text structures information or ideas into categories or hierarchies, demonstrating understanding of the information or ideas.

**RST.11-12.6**
Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, identifying important issues that remain unresolved.

or a recommendation for solving a scientific or technical problem.

**RST.9-10.9**
Compare and contrast findings presented in a text to those from other sources (including their own experiments), noting when the findings support or contradict previous explanations or accounts.

**RST.11-12.8**
Evaluate the hypotheses, data, analyses, and conclusions in a scientific or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.

**RST.11-12.9**
Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.

# Science in the Classroom AAAS

| AP Science Standards | |
|---|---|
| AP Science Practices | AP Biology Content Standards |
| **Science Practice 1 (SP1)**<br>The student can use representations and models to communicate scientific phenomena and solve scientific problems.<br><br>**Science Practice 3 (SP3)**<br>The student can engage in scientific questioning to extend thinking or guide investigations within the context of the AP course. | **Essential knowledge 2.D.1 (EK2.D.1)**<br>All biological systems from cells and organisms to populations, communities, and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.<br><br>**Essential knowledge 2.D.4 (EK2.D.4)**<br>Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis.<br><br>**Essential knowledge 3.D.3 (EK3.D.3)**<br>Signal transduction pathways link signal reception with cellular response. |

| Connections to the Nature of Science | |
|---|---|
| Vision and Change in Undergraduate Biology Education<br>Core Competencies and Disciplinary Practices | A Framework for K-12 Science Education<br>Understandings About the Nature of Science |
| **Ability to use quantitative reasoning (VC2)**<br>Biology relies on applications of quantitative analysis and mathematical reasoning.<br><br>**Ability to understand the relationship between science and society (VC6)**<br>Biology is conducted in a societal context. | **Scientific Knowledge is Based on Empirical Evidence (NS2)**<br>Science includes the process of coordinating patterns of evidence with current theory. Scientific arguments are strengthened by multiple lines of evidence supporting a single explanation.<br><br>**Science is a Way of Knowing (NS5)**<br>Science is both a body of knowledge that represents a current understanding of natural systems and the processes used to refine, elaborate, revise, and extend this knowledge. |