# Solutions for **Oncology Therapeutics Discovery**



Your Partner From Discovery Through Development



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Antibodies That Work on Your Platform **Put our experience to work for you.** Cell Signaling Technology (CST) scientists are biology, application, and therapeutic area domain experts and are here to streamline your discovery. We are passionate about science and keep up with all the latest research and new treatment approaches in oncology therapeutic areas, allowing us to offer a broad antibody portfolio against targets that could lead to new, more efficacious therapies.

# TAILOR CST PRODUCTS & SERVICES TO FIT YOUR PROJECT NEEDS

# **Quality & Reproducibility**

Reproducibility in your experiments is not a matter of chance. It is a matter of science. CST products are peer-tested in the field and over 99.5% of CST recombinant monoclonal antibodies are manufactured in-house, providing complete control over our supply chain. Our products are developed, tested, and rigorously validated across multiple applications by tenured CST scientists who understand the underlying biology. This ensures that you will always get the lot-to-lot and assay-to-assay consistency required for the lifetime of your project.

# **Assay Support & Flexibility**

Accelerate time to results with CST products and services. Your assay is only as good as your antibody is specific. CST antibodies and ready-to-use ELISA and cellular assay kits are developed with this in mind and are designed to seamlessly fit into your assay workflow and instantly answer key questions. Products are optimized across multiple applications and CST subject matter experts are available to help identify the best readout and clone to effectively assess your therapeutic efficacy and safety. Choose the ideal product for your application.

# **Companion Reagents**

CST offers a wide selection of epitope-tagged and control antibodies, secondary antibodies, detection reagents, and experimental controls as well as standard buffers and other reagents required to complete your experimental workflow. These same reagents are also used in-house for antibody validation for applications including western blotting, immunohistochemistry, flow cytometry, and immunofluorescent analysis and, therefore, work optimally with our primary antibodies. Technical support is provided by the product scientists who make the reagents and know them best.

## **Services & Customization**

Sometimes the fastest way to move your discovery forward is to have someone else do it. CST provides custom solutions that meet your specific research challenge, freeing up your time to focus on the science.

SERVICES AND CUSTOMIZED OFFERINGS	OVERVIEW
Carrier-free and Customized Formulations	Ideal for any assay or platform such as High Throughput ELISA assays, Multiplex IHC, Flow Cytometry, IHC Autostainers, and High Content Screening and Analysis
Custom Antibody Conjugation	Have our in-house experts perform the conjugations you need for your assay: fluorophores, biotin, enzymes, and more
Proteomics Analytical Services	Qualitative and quantitative protein profiling of your samples by our team of experts. Uncover proteome and post-translational modifications pre- and post-treatment
Bulk Quantities and Lot Reservation	Eliminate potential supply problems by partnering with CST to reserve a single lot or place bulk orders
Custom Peptides and Controls	CST provides the controls you need for your assay

# **Oncology Drug Development**

# **KINASE INHIBITORS**

Protein kinases are involved in a wide variety of cellular functions including cell cycle regulation and differentiation. Investigating oncogenic kinases like Bcr/Abl and K-Ras have advanced the field of cancer and led to the emergence of targeted therapies and personalized medicines as a cancer therapeutic strategy.

# **RTKS/GROWTH FACTOR RECEPTORS**

- ▶ EGFR, mutant EGFR
- ▶ ROS1, ALK
- ▶ Met
- ▶ VEGF Receptor
- PDGF Receptor



ROS1 (D4D6®) Rabbit mAb #3287: IHC analysis of paraffinembedded human lung carcinoma using #3287.

#### **INTRACELLULAR KINASES**

- ▶ B-Raf
- Ras, K-Ras, Active Ras Detection Kit
- ▶ IRAK
- ► GSK3
- ▶ c-Jun



Ras (E8N8L) XP® Rabbit mAb #67648: IHC analysis of paraffinembedded human non-small cell lung carcinoma using #67648.

#### **KINASE INHIBITION READOUTS**

- ▶ Phospho-Akt
- ▶ Phospho-p44/42 MAPK (Erk1/2)
- ▶ Phospho-p38 MAPK
- ▶ Phospho-MEK1/2
- ▶ Phospho-p70 S6 Kinase/p70 S6 Kinase



FastScan<sup>™</sup> Phospho-p44/42 MAPK (Erk1/2) MAPK (Thr202/ Tyr204) ELISA Kit #42173

# CELL CYCLE AND DNA DAMAGE RESPONSE (DDR)

Cell cycle checkpoint and DDR pathways work together to preserve genomic integrity. Perturbations in either pathway can lead to uncontrolled cell proliferation and the accumulation of mutations that drive tumorigenicity. Targeting defects in these pathways can lead to next-generation therapeutics for multiple cancer types.

## **CELL CYCLE REGULATION**

- ▶ Phospho-Rb
- ► CDK4/6, CDK2
- ▶ Cyclin D1, Cyclin E1
- ▶ PLK1



FastScan<sup>™</sup> Phospho-Rb (Ser807/811) ELISA Kit #10754

## DNA DAMAGE RESPONSE (DDR)

- ▶ Phospho-p53/p53, MDM2
- Phospho-Chk1/Chk1, Phospho-Chk2
- ▶ PARP, Rad51
- ► ATM, ATR



Phospho-Chk1 (Ser345) (133D3) Rabbit mAb #2348: Confocal IF analysis of C2C12 cells, untreated (left) or UV-treated (right), using #2348 (green). Actin filaments have been labeled with DY-554 phalloidin (red).

## SENESCENCE

- ▶ p16 INK4A
- ▶ p21 Waf1/Cip1
- ▶ Lamin B1
- β-galactosidase Assays



**p21 Waf1/Cip1 (12D1) Rabbit mAb #2947:** IHC analysis of paraffin-embedded human breast carcinoma using #2947 in the presence of control peptide.

# **CANCER METABOLISM**

The cancer metabolism field has evolved from describing the metabolic features of cancer cells to understanding how cancer cells are metabolically reprogrammed and how that influences migration, invasion, and metastasis. Oncology therapeutics targeting metabolic pathways could reduce cancer growth and provide additional combination therapy options.

# **GLUCOSE METABOLISM**

- ▶ Phospho-PKM2/PKM2
- ▶ Phospho-IGF-I Receptor/IGF-I Receptor
- Phospho-Insulin Receptor β/Insulin Receptor β
- Phospho-GSK-3β/GSK-3β
- ▶ Glut1

PKM2 (D78A4) XP® Rabbit mAb #4053: IHC analysis of paraffin-embedded human lung carcinoma using #4053.

# FATTY ACID AND AMINO ACID METABOLISM

- Phospho-Acetyl-CoA Carboxylase/ Acetyl-CoA Carboxylase
- ▶ Perilipin-1
- Arginase-1
- ► ASS1
- ▶ Phospho-BCKDH



Phospho-Acetyl-CoA Carboxylase (Ser79) (D7D11) Rabbit mAb #11818: IHC analysis of paraffin-embedded human breast carcinoma using #11818.

#### AUTOPHAGY

- ▶ LC3B
- ▶ Phospho-mTOR/mTOR
- ► Atg13
- ▶ ULK1
- ▶ SQSTM1/p62



LC3B (E502K) Mouse mAb #83506: Confocal IF analysis of HCT 116 cells treated with Chloroquine #14774 (50  $\mu$ M, overnight) using #83506 (green). Actin filaments were labeled with  $\beta$ -Actin (13E5) Rabbit mAb #4970 (red) and nuclei were labeled with DAPI #4083 (blue).

# **TRANSFORMATION/TUMORIGENESIS**

Epithelial-mesenchymal transition (EMT) is a cellular program known to play a crucial role in malignant progression, promoting metastasis and therapy resistance. Novel therapeutics target the activation of EMT to reduce the spread of cancer throughout the body and improve patient outcomes.

## TRANSCRIPTION FACTORS/ CO-ACTIVATORS



## SIGNALING PATHWAYS

- Phospho-SMAD2/3 ELISA, TGF-β
- Wnt/β-Catenin
- ▶ YAP/TAZ, LATS1
- DLL3, Notch1/2/3



## **KEY BIOMARKERS**

- ▶ Vimentin
- ► E-Cadherin
- Vinculin
- ▶ Fibronectin
- ▶ a-Smooth Muscle Actin



Vimentin (D21H3) XP<sup>®</sup> Rabbit mAb #5741: IHC analysis of paraffin-embedded human breast carcinoma using #5741.

# **TARGETING APOPTOSIS**

Apoptosis evasion is one of the hallmarks of cancer. Targeting an apoptotic pathway to trigger cell death in cancer cells is a particularly intriguing approach that could lead to universal cancer therapeutics that are not specific to a cancer type.

# **INTRINSIC PATHWAY**

- ▶ BcI-2, BcI-xL, McI-1
- ▶ Bax, Bim, Bad
- ▶ Puma, Noxa



Bcl-xL (54H6) Rabbit mAb #2764: IHC analysis of paraffinembedded human lung carcinoma, using #2764.

# **EXTRINSIC PATHWAY**

- ▶ TRAIL, DR4, DR5
- ▶ TNF, TNF-R1, TNF-R2

▶ FasL, Fas



DR5 (D4E9) XP® Rabbit mAb #8074: Confocal IF analysis of HT-1080 cells using #8074 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

# **CASPASE ACTIVATORS**

- ▶ Smac/Diablo
- ► XIAP, c-IAP1, c-IAP2
- ► Survivin, Livin



Survivin (7164B7) Rabbit mAb #2808: IHC analysis of paraffin-embedded human transitional epithelial carcinoma of the bladder using #2808.

# **EPIGENETIC MODULATION**

Aberrent epigenetic processes and genetic mutations both contribute to oncogenesis; however, most cancer therapies focus on targeting genetic abnormalities. Epigenetic therapies that reprogram neoplastic cells to behave more like normal cells provide an alternative therapeutic approach and are likely to be the most efficacious when used in combination with other anticancer therapies.

## METHYLATION AND DEMETHYLATION INHIBITORS

- ▶ Histones Regulators: Ezh2, LSD1, H3K27me3
- DNA Regulators: DNMTs, TETs, 5-Methylcytosine (5-mC), 5-Hydroxymethylcytosine (5-hmC)
- ▶ RNA Regulators: N6-Methyladenosine (m6A), METTL3, FT0



Ezh2 (D2C9) XP<sup>®</sup> Rabbit mAb #5246: IHC analysis of paraffin-embedded human lymphoma using #5246.

# ACETYLATION AND DEACETYLATION INHIBITOR

▶ Histones Regulators: CBP, p300, H3K27ac, HDACs

# **HISTONE MUTATIONS**

 Histones: H3K27M, H3K36M, H3K9M, H3K27me3, H3K36me3, H3K9me3



**p300 (D8Z4E) Rabbit mAb #86377:** IHC analysis of paraffinembedded human squamous cell lung carcinoma using #86377.



Histone H3 (K9M Mutant Specific) (E4N7V) Rabbit mAb #54005: IHC analysis of parafin-embedded histone H3 K9M mutant mouse small intestine using #54905. (Tissue courtesy of Dr. Aaron Huebner, Hochedlinger Lab at Massachusetts General Hospital, Boston, MA.)

# Immuno-oncology Drug Development

Immuno-oncology (I/O) is a paradigm-shifting treatment approach that stimulates a patient's own natural immune system to eliminate cancer cells instead of targeting the cancer cells directly. It is more efficacious in some patients and can maintain a better quality of life compared to other approaches including surgery, chemotherapy, and targeted therapies. The I/O drug development landscape continues to evolve as the interplay between the immune system and cancer cells is further characterized.

# IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT

Tumor growth and survival are influenced by malignant cells and signals from the endothelial cells, stromal fibroblasts, and immune cells that make up the tumor microenvironment (TME). The degree of tumor infiltration by cytotoxic T-cells as well as complex interactions with endothelial cells and fibroblasts can predict and modulate clinical outcomes.

#### **IMMUNE CELL INFILTRATION**

- ► CD3
- ► CD8a
- ► CD68
- ▶ CD19
- ► CD11c
- 000

**CD8α (D8A8Y) Rabbit mAb #85336:** IHC analysis of paraffinembedded human Crohn's diseased colon using #85336.

#### **IMMUNE CELL FUNCTION**

- ▶ FoxP3
- ▶ HLA-DR
- ▶ CD86
- ▶ IL-1β
- ▶ IL-8



CD86 (E2G8P) Rabbit mAb #91882: IHC analysis of paraffin-embedded human lung adenocarcinoma using #91882 performed on the Leica BOND Rx.

## THERAPEUTIC TARGETS

- ▶ CD47
- ▶ CD73
- ▶ SIRPα
- CSF-1



CD47 (D307P) Rabbit mAb #63000: IHC analysis of paraffin-embedded human urothelial carcinoma using #63000 performed on the Leica BOND Rx.

# MULTIPLEX IHC ENABLEMENT

Gold-Standard IHC Validation Enables Multiplex Discovery



Multiplex IHC analysis of paraffin-embedded malignant melanoma on the CODEX platform using Granzyme B (D6E9W) Rabbit mAb #46890, shows the widespread labeling of infiltrating immune cells in this sample, along with other markers.

#### **Reliably Correlate Protein Expression with Genomic Data and IHC**



Serial sections of each NSCI C specimen were stained with antibodies for each of the noted proteins. Tumor Ras G12C genotype of each sample (A-C) obtained from SNV analysis is indicated (+/-) on the left. An additional single serial section was simultaneously stained with DNA-barcoded antibodies. Normalized digital counts from the nCounter analysis for each protein are shown from left to right, color coded to each sample (A-C).

# Immuno-oncology Drug Development

# **IMMUNE CHECKPOINTS**

T cell-targeted immunomodulators that block immune checkpoints like PD-1, PD-L1, and CTLA-4 have revolutionized how we approach cancer treatment. These therapies have significantly improved outcomes for some patients with late-stage cancers.

#### **CHECKPOINT THERAPY TARGETS**

- ▶ PD-1
- ▶ PD-L1
- ▶ TIGIT
- ▶ B7-H3
- ► ICOS

TIGIT (E5Y1W) XP® Rabbit mAb #99567: IHC analysis of paraffin-embedded human non-small cell lung carcinoma using #99567.

# **T CELL EXHAUSTION**

- ▶ PD-1
- ► TOX
- ► TCF1
- TIM-3LAG3



Tox/Tox2 (E6I3Q) Rabbit mAb #73758: IHC analysis of paraffin-embedded human colon carcinoma using #73758 performed on the Leica<sup>®</sup> BOND<sup>™</sup> Rx.

# **T CELL ACTIVITY**

- ► IFN-γ
- ▶ TNF-α
- Granzyme B
- Cytotoxicity Assay
- Proliferation Assay



IFN-y (Alexa Fluor® 488 Conjugate)

IFN-y (D3H2) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate) #12942: Flow cytometric analysis of human peripheral blood mononuclear cells treated with TPA, Ionomycin and Brefeldin A using #12942. Co-stained with CD3 (UCHT1) Mouse mAb (APC Conjugate) #19881.

# **INNATE IMMUNE STIMULATORY**

Activation of the innate immune system can counteract the pathways triggered by cancer cells to evade the immune system. Further insights into these mechanisms will potentially lead to novel I/O therapeutics that can lead to long-term clinical benefits in combination with other therapeutic approaches.

## INFLAMMASOME

- ▶ NLRP3
- ▶ Caspase-1/Cleaved Caspase-1
- ► IL-1β/Cleaved IL-1β
- ► ASC/TMS1
- ▶ IL-18



**IL-1β (3A6) Mouse mAb #12242:** HC analysis of paraffinembedded human large intestine (ulcerative chronic colitis of the rectum) using #12242.

## STING

- ▶ cGAS
- ▶ Phospho-STING/STING
- Phospho-TBK1/TBK1
- Phospho-IRF-3/IRF-3



Phospho-STING (Ser366) (E9A9K) Rabbit mAb #50907: Flow cytometric analysis of TPA differentiated THP-1 cells that have been activated to phosphorylate STING (green line) vs unactivated (blue). Isotype controls are shown by dashed lines.

## TLR

- ▶ Phospho-IkBa/IkBa
- ▶ Phospho-IKKa/β/IKKa/β
- ▶ Phospho-p65/p65
- ▶ Phospho-TBK1/TBK1
- ▶ Phospho-IRF-3/IRF-3



NF-κB p65 (D14E12) XP<sup>®</sup> Rabbit mAb #8242: IHC analysis of paraffin-embedded human chronic cholecystitis using #8242.

# **IMMUNE CELL ACTIVATION ASSAYS**

Immune cell activation readouts can be used to assess the activation of the innate and adaptive immune system when determining the efficacy of novel immuno-oncology therapeutics.

## **T CELL SIGNALING**

- ▶ Phospho-SLP-76 (Ser376)
- ▶ Phospho-NF-kB (Ser536)
- ► HPK1
- ▶ Phospho-Zap-70 (Tyr319)
- ▶ Phospho-Erk (Thr202/Tyr204)
- ▶ Phospho-SHP-2 (Tyr580)



PathScan<sup>®</sup> Phospho-SLP-76 (Ser376) Sandwich ELISA Kit #78222



 
 Phospho-SLP-76 (Ser376) (Alexa Fluor® 647 Conjugate)
 Phospho-SLP-76 (Ser376) (Alexa Fluor® 647 Conjugate)

 Phospho-SLP-76 (Ser376) (E3G9U) XP® Rabbit mAb (Alexa Fluor® 647 Conjugate) #61762: Flow cytometric analysis comparing human peripheral blood mononuclear cells untreated or treated with cross-linked anti-CD3 plus anti-CD28.

#### **B CELL SIGNALING**

- Phospho-Syk (Tyr525/526)
- Phospho-Btk (Tyr223)
- ▶ Phospho-Src Family (Tyr416)
- Phospho-CD79A (Tyr182)
- ▶ Phospho-SHP-1 (Tyr564)



PathScan® Phospho-Btk (Tyr223) Sandwich ELISA Kit #23843



Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb (PE Conjugate) #6485: Flow cytometric analysis comparing treated (green) vs. untreated (blue) Ramos cells.

#### **CYTOKINE RESPONSE**

- Phospho-STAT3 (Tyr705)
- ▶ Phospho-STAT5 (Tyr694)
- ▶ Phospho-STAT6 (Tyr641)
- ▶ Phospho-STAT1 (Tyr701)
- Phospho-Jak2 (Tyr1007/1008)
- Phospho-Jak1 (Tyr1034/1035)



FastScan<sup>™</sup> Phospho-Stat1 (Tyr701) ELISA Kit #40716



Phospho-Stat3 (Tyr705) Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145: Flow cytometric analysis comparing treated (green) vs. untreated (blue) U266 cells using #9145 (solid lines) or isotype control (dashed lines).



PathScan<sup>®</sup> Phospho-NF-кB p65 (Ser536) Sandwich ELISA Kit #7173



PathScan® Phospho-Syk (Tyr525/526) Sandwich ELISA Kit #7970



PathScan<sup>®</sup> Phospho-Stat3 (Tyr705) Sandwich ELISA Kit #7300

# **Adoptive Cell Therapy and Cell Viability Assays**

# **ADOPTIVE CELL THERAPY**

Adoptive cell therapies are an immunotherapeutic approach that utilizes tumor-infiltrating lymphocytes or gene-modified T cells expressing novel T cell receptors (TCR) or chimeric antigen receptors (CAR) to eradicate tumor cells. Further development goals for this treatment approach include improving the anti-tumor efficacy, reducing associated toxicity, and realizing allogenic potential.

# MONITORING CAR-T TARGET EXPRESSION

- ► TNFRSF17/BCMA
- ▶ CD19
- ▶ IL-13RA2
- ▶ CD20
- ▶ CD22



TNFRSF17/BCMA (E6D7B) Rabbit mAb #88183: IHC analysis of paraffin-embedded human normal colon using #88183.

# T CELL PHENOTYPING

- ► CD4
- ► CD8
- ▶ PD-1







TIM-3 (Alexa Fluor® 488 Conjugate) TIM-3 (D5D5R") XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #54669: Flow cytometric analysis of primary CD4\* T cells (green, positive) and Jurkat cells (blue, negative)

## T CELL FUNCTIONAL ANALYSIS

- Cytotoxicity assay
- Proliferation assay
- ▶ Granzyme B
- ▶ IFN-v
- ▶ TNF-α



Cell Proliferation Tracer Kit (Fluorometric, Violet 450) #48444: Live human peripheral blood mononuclear cells were labeled with the kit comparing treatment to induce cellular division (green) vs. untreated (solid blue line). Unstained cells were used as a control (dashed line). Multiple peaks equate to multiple rounds of cellular division (proliferation) following treatment.

# **CELL VIABILITY ASSAYS**

Cell viability is an indication of the percentage of healthy cells within a sample. Biological readouts for cell viability are used to assess the safety and efficacy of therapeutics developed to inhibit proliferation or promote apoptosis in cancer cells.

## PROLIFERATION

- ▶ Ki-67
- ▶ PCNA
- ▶ Phospho-Histone H3
- BrdU Proliferation Assay



**Ki-67 (8D5) Mouse mAb #9449:** IHC analysis of paraffinembedded human breast carcinoma using #9449.

#### **CELL DEATH**

- Apoptosis: Cleaved Caspase-3 ELISA, Cleaved PARP ELISA, Annexin V Assay, Mitochondrial Membrane Potential Assay
- ▶ Necroptosis: RIP/Phospho-RIP ELISA, Phospho-MLKL
- Pyroptosis: NLRP3, Cleaved Gasdermin, Cleaved Caspase-1



PathScan® Phospho-RIP (Ser166) Chemiluminescent Sandwich ELISA Kit #88918

## VIABILITY

- ▶ XTT Cell Viability Assay
- ▶ Resazurin Cell Viability Assay



XTT Cell Viability Kit #9095: C2C12 cells were seeded at varying density in a 96-well plate and incubated overnight. The XTT assay solution was added to the plate and cells were incubated. The absorbance at 450 nm was measured at 1.0, 2.0, 3.0, 4.0, and 5.0 hours.

# Solutions Across the Drug Development Continuum

Industry-leading & Aligned to Your Workflow

# TARGET ID AND MOA STUDIES

Proteomic Services leveraging MS technology

# **TARGET VALIDATION**

- ▶ Western Blot
- ▶ IHC and Multiplex IHC
- ► Flow Cytometry

# FLEXIBLE PACKAGING

- Trial Sizes
- ▶ Bulk Quantities

# SCREENING & LEAD OPTIMIZATION

# IDENTIFY PRIMARY AND SECONDARY ENDPOINTS

▶ ELISA, HCS, Multiplex IHC

# PLATFORM COMPATIBILITY

- ► ELISA: MSD, AlphaLISA, HTRF, Quanterix
- ► Multiplex IHC: Akoya, Hyperion, Nanostring
- Automated WB (e.g., ProteinSimple)

# CUSTOM

- Matched antibody pairs (ELISA)
- Antibody conjugation services

# PRECLINICAL SAFETY & VALIDATION

INDUSTRY-LEADING IHC validation for biomarker identification and detection

# READY-TO-GO ELISA KITS

to key readouts and translational biomarkers

# **IHC AUTOSTAINER COMPATIBLE**

# Antibodies That Work on YOUR Platform

# CST antibodies are routinely used with a multitude of platforms and technologies

PLATFORMS	APPLICATION	CONJUGATE TYPE <sup>.</sup>	PRODUCT FORMAT
TR-FRET/LANCE/HTRF AlphaLISA/Screen MSD Quanterix (Simoa) Luminex	High Throughput ELISA	Fluorophores Lanthanides Biotin Beads	BSA- and Azide-free Formulation Matched Antibody Pairs Optimized Concentration
CyTOF/Helios Hyperion MIBI	Flow Cytometry Mass Cytometry/IMC Multiplex IHC	Metal lons	BSA- and Azide-free Formulation Optimized Concentration
AKOYA (CODEX, OPAL) ULTIVUE IMMUNOSABER NANOSTRING	Multiplex IHC	Oligonucleotides Fluorophores	
IHC Autostainers	IHC	N/A	
ProteinSimple	Automated WB	N/A	
High Content Screening & Analysis	IF Imaging	Fluorophores	BSA- and Azide-free Formulation

\*Some conjugates directly available for purchase from CST (other conjugate types may be proprietary)



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†U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.

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